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Effectiveness of XP-Endo Finisher in the reduction of bacterial load in oval-shaped root canals

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Abstract: This study investigated the effectiveness of XP-Endo Finisher (XPF) associated with XP-Endo Shaper (XPS) or Reciproc Blue (RB) files in reducing bacterial load in oval-shaped root canals (RC) during chemomechanical preparation (CMP) using 0.9% saline solution (NaCl) or 2.5% sodium hypochlorite (NaOCl). Eighty mandibular incisors with single oval-shaped RC were contaminated with *Enterococcus faecalis*. The teeth were randomly assigned to eight experimental groups (n = 10) according to the CMP, as follows: G1: XPS, G2: XPS + XPF, G3: RB, and G4: RB + XPF. CMP was performed with NaCl or NaOCl. The reduction of bacterial load was assessed by colony-forming unit count before (S1) and after (S2) CMP. Data normality was verified by using Shapiro-Wilk test. ANOVA, Tukey's test, and Bonferroni post-hoc test were used at a 5% significance level. Culturable bacteria were present in all S1 samples ($p>0.05$). All instrumentation techniques were effective in reducing bacterial load, irrespective of the irrigating solution ($p < 0.05$). With the use of NaCl, RB was more effective than XPS ($p = 0.035$). With the use of NaOCl, XPS and RB presented similar effectiveness ($p = 0.779$). XPF enhanced the bacterial reduction of both systems tested ($p < 0.05$). The use of NaOCl improved the CMP, irrespective of the instrumentation technique used ($p < 0.05$). In conclusion, XPS and RB files are effective in reducing bacterial levels in oval-shaped RC. The use of XPF as a method of agitation of the irrigating solution improved the cleaning efficiency of both file systems tested. Mechanical preparation performed with saline solution decreased culturable bacteria from the root canal, but antimicrobial substances such as NaOCl should be used to achieve a significantly better disinfection.

Keywords: *Enterococcus faecalis*; Sodium Hypochlorite; Biofilms; Endodontics.

Introduction

The main goal of endodontic therapy is to prevent or eliminate apical periodontitis by means of cleaning, shaping, disinfecting, and filling the root canal system. The removal of microorganisms is considered the most important step in root canal therapy.[1,2,3] Anaerobic bacteria, particularly gram-negative species, have been linked to the signs and symptoms of periapical disease. Facultative gram-positive bacteria, such as *Enterococcus*

faecalis, have also been detected in infected root canals and may be related to failure of root canal treatment, showing capacity to survive intracanal procedures.[1,4,5]

Mechanical preparation of the root canals is recognized as one of the most important stages in root canal treatment. Although many advances in file designs have been made in the last decades, irrespective of the technique used, several studies have shown that complete removal of organic tissues/debris[6,7,8] and bacteria[9,10] is not commonly attained.

Several single-file techniques for root canal instrumentation have been introduced recently. Literature has shown no differences in the elimination of bacteria from root canals by comparing single-file to multi-file systems.[11,12] Reciproc Blue (VDW, Munich, Germany) is a system which uses reciprocating motion[13] and has a newly developed alloy obtained through a thermomechanical manufacturing process, resulting in a specific oxide surface layer.[13] XP-Endo Shaper (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland) is also a single-file instrument, but it uses rotary motion, with the purpose of cleaning and shaping the root canals.[14] It is manufactured from a MaxWire® alloy and has flexibility and fatigue strength, being able to expand and to be advanced easily and quickly into the canal at a reduced risk of torsional fatigue.[14]

It is known that most of the available systems fail to perform adequate cleaning and shaping, leaving untouched areas within the root canal.[6,8] Moreover, considering all the challenges involved during endodontic treatment (*i.e.*, isthmuses, intercanal communications, curvatures, and oval-shaped canals), a supplementary cleaning approach associating mechanical preparation with root canal irrigants (*i.e.*, sodium hypochlorite) and new irrigation technologies may help in everyday clinical practice.[15]

XP-Endo Finisher (FKG Dentaire SA) has been recently introduced as an adjunctive approach to improve the effectiveness of irrigation and disinfection during chemomechanical preparation (CMP). Apparently, this instrument has the potential to be used as an additional therapeutic procedure in order to maximize bacterial removal.[16] Changes in this newly developed file were proposed to increase its effectiveness in touching larger areas of the root canal walls and dislodging biofilms that remain after CMP. Thus, the present study aimed to investigate the effectiveness of XP-Endo Finisher associated with XP-Endo Shaper or Reciproc Blue files in reducing bacterial load in oval-shaped root canals during CMP using 0.9% saline solution (NaCl) or 2.5% sodium hypochlorite (NaOCl). The null hypothesis was that there are no differences in bacterial reduction with the additional use of XP-Endo Finisher.

Methodology

Specimen selection and preparation

This study was revised and approved by the Research Ethics Committee of the Piracicaba Dental School, State University of Campinas – UNICAMP (CAAE: 48274215.6.0000.5418). The specimens were selected according to Silva et al.[17] Briefly, a sample of 80 mandibular incisors was selected from a pool of teeth extracted for reasons not related to this study and stored in 0.1% thymol solution at 5ºC. Digital radiographs were taken in the buccolingual and mesiodistal directions to select only teeth with single oval-shaped root canals, with a cross-section diameter ratio ≥ 2.5:1 at 5 mm from the apex. After acquiring digital images of each specimen, the angle of curvature was measured with the aid of image analysis software (AxioVision 4.5; Carl Zeiss Vision, Hallbergmoos, Germany). Teeth with root curvature < 10° and initial apical size equivalent to a #15 K-file (Dentsply Sirona, Ballaigues, Switzerland) were included in the present study. Pairs of teeth were selected on the basis of similar radiographic root canal morphology, and each tooth from each pair was randomly assigned to each experimental group. Exclusion criteria were as follows: presence of dental caries, fractures, immature apex, multi-rooted teeth, no calcifications, no internal or external root resorptions, no dental posts or prosthetic crowns, and no prior endodontic treatment.

Conventional access cavities were prepared by using round burs (KG Sorensen, Cotia, Brazil) and Endo-Z burs (Dentsply Maillefer, Ballaigues, Switzerland). A glide path was created by introducing a stainless steel of size #15 K-file (Dentsply, Maillefer, Ballaigues, Switzerland) up

to the working length (WL), which was established at the apical foramen.

All teeth were instrumented at the apical foramen up to a size of #25 K-file in alternating rotation motions under continuous irrigation with 10 mL of distilled water. The smear layer was removed by using 17% EDTA for 3 minutes followed by copious irrigation with 5 mL of distilled water. Irrigation was performed by using a NaviTip needle (Ultradent, South Jordan, UT, USA) placed as much apically as possible to ensure that the irrigants could reach the entire extent of the canal. The teeth were then immersed in brain heart infusion broth (BHI, Difco, Kansas City, MO, USA), ultrasonically activated for 1 minute to release entrapped air and allow penetration of the culture medium into root canal irregularities. Next, the teeth were sterilized in an autoclave for 20 minutes at 121°C.

Contamination with *Enterococcus faecalis*

Enterococcus faecalis (ATCC 29212) was used to infect the root canals. The specimen contamination protocol was adapted from Andrade et al.[18] Briefly, *E. faecalis* was reactivated in BHI broth and maintained at 37°C for 24 hours. The bacterial culture was transferred to another BHI flask and incubated for another 24 hours to achieve exponential growth. This culture was adjusted to McFarland standard #1 (3×10^8 CFU/mL) by means of a spectrophotometer (Bel Photonics do Brasil Ltda, Osasco, Brazil). First, 800 μL of sterilized BHI was inserted in each Eppendorf tube (Axygen Scientific, Union City, USA) containing the specimen. Activation was performed in an ultrasonic bath for 15 minutes to allow for maximum penetration of the culture medium into the dentinal tubules prior to bacterial contamination. Contamination using the standardized inoculum was performed for 10 days, with centrifugation on alternate days to allow bacterial penetration into the root canal system. Following adjustment of the *E. faecalis* culture, it was incubated again at 37°C for 7 hours in order to achieve exponential growth. The inoculum (800 μL) was inserted into Eppendorf tubes with the specimens and centrifuged (Eppendorf 5417R centrifuge, Eppendorf, Hamburg, Germany) in sequence at 1,400, 2,000, 3,600, and 5,600 g in two five-minute cycles for each speed. The inoculum was renewed at each centrifugation cycle. Following the eight centrifugation cycles, sterilized BHI broth was inserted into Eppendorf tubes, agitated in a vortex mixer (Vortex-mix, Edison, USA), and incubated at 37 °C for 24 hours (This protocol was repeated on days 1, 3, 5, 7, and 9). Following incubation (day 2), the samples were agitated again in a vortex mixer for 10 seconds, and the inoculum from the Eppendorf tubes was discarded. One milliliter of sterilized BHI broth was inserted, followed by a centrifugation cycle of 3,600 g for 5 minutes at 25°C, and the Eppendorf tubes were incubated again at 37°C for 24 hours (This protocol was repeated on days 2, 4, 6, and 8). On day 10, the samples were removed from Eppendorf tubes, excess culture medium was removed, and the external root surfaces wiped with sterile gauze. Figure shows bacterial penetration into the dentinal tubules by scanning electron microscopy (SEM). A pilot study showed that 10 day was enough to contaminate the root canals using the contamination protocol.

The apical foramen of each experimental tooth was sealed with a fast-setting epoxy resin to prevent apical bacterial leakage and to create a closed-ended channel that produces the vapor lock effect.[19] The teeth were mounted vertically up to the cervical region in blocks made of silicone impression material

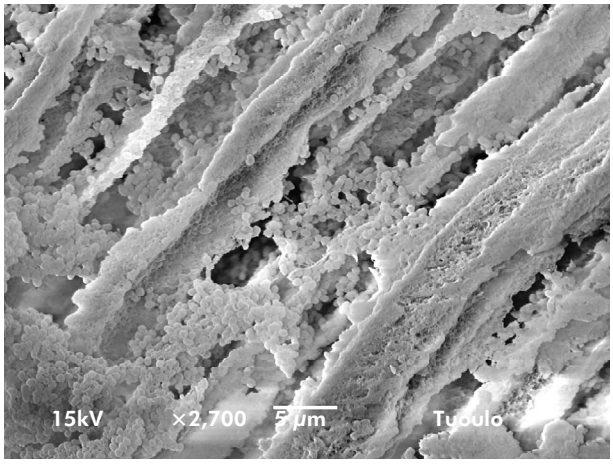

Figure. Scanning electron microscopic image of *Enterococcus faecalis* biofilm formation in the root canal walls and within the dentinal tubules.

(President Jet; Coltene AG, Cuyahoga Falls, OH, USA). The tooth crown, including the pulp chamber walls, and the silicone surface were disinfected with 2.5% NaOCl, followed by inactivation with 10% sodium thiosulfate.

Sampling and culture procedures

Initial bacteriological samples (S1) were taken from all canals, irrigated with 1 mL of sterile saline solution, before CMP.

Bacteria were recovered by moving a sterile precurved stainless steel size #25 Hedstroem file (Dentsply-Maillefer, Ballaigues, Switzerland) three times along the dentin wall from apical to coronal thirds and then placing the file into a sterile tube containing 1 mL of BHI broth for bacterial sampling. Next, a sterile paper point (Dentsply-Maillefer, Ballaigues, Switzerland) was introduced into the full working length of the canal and retained in position for 60 seconds. Other two paper points were consecutively introduced and then transferred to a sterile tube as described above.

The method for counting the colony-forming unit per milliliter (CFU/mL) has been previously published by Marinho et al.[12] Briefly, the Eppendorf tubes containing the root canal samples were shaken thoroughly for 60 seconds (Vortex; Marconi, Piracicaba, Brazil) and then serial 10-fold dilutions were made up to 10^{-4} in tubes containing BHI broth. By using sterile plastic spreaders, 50 µL of each serial dilution was plated onto 5% defibrinated sheep blood BHI agar plates and subjected to bacterial count after 48 hours of incubation at 37°C. The tubes containing the serial dilutions were kept in the incubator at 37°C for 7 days to confirm microbial growth.

Chemomechanical preparation protocol

An experienced operator performed the CMP of all specimens. During the experiment, only one instrument was used for each tooth. The teeth were randomly assigned to eight groups (n = 10) by using a computer algorithm (http://www.ramdom.org) and according to the disinfection protocol.

All procedures were performed at 37°C inside a laminar flow cabinet containing a heater (800-Heater; PlasLabs, Lansing, USA). In all groups, the teeth were irrigated with 0.9% NaCl or 2.5% NaOCl at 37°C by using a syringe and a 30-gauge NaviTip needle (Ultradent, South Jordan, USA). The irrigation needle was placed 2 mm short from the WL. The total volume of irrigating solution used in all specimens was standardized to 20 mL during CMP, according to Marinho et al.[12] Next, the specimens irrigated with 2.5% NaOCl were inactivated by using 5 mL of 5% sodium thiosulfate, whereas in the 0.9% NaCl groups, the specimens were irrigated with 5 mL of 0.9% NaCl to standardize the total volume of solution used during the experiment. A final flush with 5 mL of 0.9% NaCl was performed in both groups. The irrigating solution was evacuated during CMP by using 0.36-mm capillary tips (Ultradent Products INC, South Jordan, USA). The contact time between the irrigants and the root canal walls was 4 minutes (time for CMP). In the groups in which XP-Endo Finisher was used, an additional minute was necessary. In the groups without agitation, irrigating solutions were kept in contact for the same period (1 minute) to standardize the total amount of time (5 minutes).

XP-Endo Shaper Group: Twenty teeth were instrumented with XP-Endo Shaper according to the manufacturer's recommendations. XP-Endo Shaper was used until reaching the WL by applying up-and-down movements. These movements were repeated three times until the WL was reached. After reaching the WL, the XP-Endo Shaper instrument was cleaned and the canal was irrigated with 0.9% NaCl (n = 10) or 2.5% NaOCl (n = 10) and another 10 gentle movements were performed at the WL.

XP-Endo Shaper + XP-Endo Finisher Group: Twenty teeth were instrumented with XP-Endo Shaper as described before and an additional approach with XP-Endo Finisher was performed according to the manufacturer's recommendations. XP-Endo Finisher was used in the canal for 1 minute at 800 rpm and 1 Ncm up to the WL and in slow up-and-down movements at 8-mm length.

Reciproc Blue Group: Twenty teeth were instrumented with Reciproc Blue R25 file. The instrument was gently inserted into the cervical third with an in-and-out pecking motion, with a light apical pressure in a reciprocating motion ('RECIPROC ALL') powered by an electric motor (VDW Silver GmbH, Munich,

Germany) with low amplitude until the WL was reached. After three in-and-out movements, the instrument was removed from the canal and cleaned.

Reciproc Blue + XP-Endo Finisher Group: Twenty teeth were instrumented with Reciproc Blue R25 file as mentioned above and an additional approach with XP-Endo Finisher was performed as described before.

After CMP, a post-instrumentation sample (S2) from all specimens was performed as described above.

Statistical analysis

An initial screening for data normality was performed by using the Shapiro-Wilk test. Comparison between the experimental groups was carried out by ANOVA and post-hoc Tukey's tests. For comparison between the samples within each group, the repeated-measures ANOVA and Bonferroni post-hoc tests were used. Alpha-type error was set at the 5% significance level ($\alpha = 0.05$). The resulting data were statistically analyzed by using SPSS for Windows (Chicago, IL, USA).

Results

Table provides an overview of the amount of culturable bacteria (CFU/mL) between the groups before (S1) and after (S2) CMP. Culturable bacteria were present in all S1 samples (80/80) ($p > 0.05$).

All instrumentation techniques were effective in reducing bacterial load, irrespective of the irrigating solution used ($p < 0.05$).

After CMP using 0.9% NaCl as irrigating solution, Reciproc Blue was more effective than XP-Endo Shaper ($p = 0.035$). However, the association with XP-Endo Finisher improved the cleaning efficacy of both file systems ($p = 0.239$). After CMP (S2) using 2.5% NaOCl as irrigating solution, XP-Endo Shaper and Reciproc Blue presented similar effectiveness in root canal disinfection ($p = 0.779$). Nevertheless, XP-Endo Finisher enhanced bacterial reduction in both systems ($p < 0.05$).

The use of 2.5% NaOCl improved CMP, irrespective of the instrumentation technique used ($p < 0.05$).

Discussion

Cross-sectional root canal configurations have been classified as round, oval, long oval, flattened, or irregular canals. Oval canals have a buccolingual diameter twice greater than the mesiodistal diameter, whereas long oval canals have a buccolingual diameter twice to four times greater than the mesiodistal diameter.[20,21,22] Long oval canals occur in the apical portion in about 25% of cases and in some groups of teeth such as mandibular incisors and maxillary second premolars; however, their prevalence is greater than 50%.[23] In the distal roots of mandibular molars, the prevalence is 25 to 30%.[21]

Oval-shaped canals were used in this study because they represent a challenge to clinicians.[6,8,24,25,26] This root canal configuration possesses areas difficult to be shaped, cleaned, and filled, which increases

Table. Mean (± SD) of CFU/mL before and after chemomechanical preparation using 0.9% NaCl and 2.5% NaOCl in the different groups.

Variable	0.9% NaCl		2.5% NaOCl	
	Before CMP	After CMP	Before CMP	After CMP
XP-Endo Shaper	215800 (± 84,256)^{Aa}	681.2 (± 118,700)^{Ab1}	203600 (± 77,882)^{Aa}	21.3 (± 15,780)^{Ab2}
XP-Endo Shaper + XP-Endo Finisher	150500 (± 29,737)^{Aa}	181 (± 46,540)^{Cb1}	175200 (± 42,949)^{Aa}	2.6 (± 1,075)^{Bb2}
Reciproc Blue	213300 (± 80,465)^{Aa}	579.5 (± 55,110)^{Bb1}	190000 (± 8,069)^{Aa}	16.6 (± 15,420)^{Ab2}
Reciproc Blue + XP-Endo Finisher	211700 (± 55,140)^{Aa}	249.5 (± 78,830)^{Cb1}	193500 (± 4,378)^{Aa}	2.5 (± 1,958)^{Bb2}

$P < 0.05$ Means followed by different letters indicate statistically significant differences. Vertical uppercase letters show the comparison of the different groups using the same irrigating solution at the same time. Horizontal lowercase letters show the comparison within the same group before and after CMP. Different numbers represent statistically significant differences of irrigants used in the same technique and after CMP.

bacterial load and, consequently, the potential failure of root canal therapy.

To the best of our knowledge, this is the first study to investigate the effectiveness of XP-Endo Finisher associated with XP-Endo Shaper or Reciproc Blue files in reducing bacterial load in oval-shaped root canals during CMP with 0.9% saline solution (NaCl) or 2.5% sodium hypochlorite (NaOCl). The inert irrigating solution (NaCl) was used to test whether XP-Endo Shaper, Reciproc Blue, and XP-Endo Finisher were able to provide a satisfactory mechanical preparation, whereas NaOCl was chosen for being the most widely used root canal irrigant in endodontic practice.

Previous works in the literature have used *ex vivo* models[12,16] and *E. faecalis* contamination to evaluate the effectiveness of different protocols for root canal cleaning.[12] In the present study, culture procedures revealed the presence of culturable bacteria in 100% of the initial samples (S1), thus validating the contamination protocol. Despite the limitations of sampling biofilm with a combination of sterile endodontic files/paper points, this is a well-established protocol.[5,27,28]

All instruments used in this study were effective in reducing bacterial load in oval-shaped root canals, irrespective of the irrigating solution used. This means that the instruments have proper mechanical characteristics. When 0.9% NaCl was used as root canal irrigant, Reciproc Blue was more efficient than XP-Endo Shaper, probably because of its design, which favors a more robust touch of the root canal walls, dislodging biofilms from dentin and allowing their removal during irrigation. The use of a supplementary approach (XP-Endo Finisher) increased the cleaning efficacy of XP-Endo Shaper and Reciproc Blue systems. achieving the greatest reduction in the 2.5% NaOCl group. This emphasizes the need of using antimicrobial substances during CMP for microbial control.

CMP performed with 2.5% NaOCl provided a significantly greater reduction of bacterial load compared to 0.9% NaCl, irrespective of the instrument used, which is corroborated by Rodrigues et al.[29] This result was not unexpected as NaOCl possesses antimicrobial activity and ability to dissolve organic tissues.[30]

In the current study, XP-Endo Finisher was successfully used to lower the levels of *E. faecalis* counts by agitation of the irrigating solutions. XP-Endo Finisher (25/.00) is manufactured from a MaxWire® alloy, and according to the manufacturer, it is straight in its martensite phase when cooled, changing to the austenite phase when exposed to body temperature (37 °C). XP-Endo Finisher has a unique spoon-shaped design with a 10-mm length from the tip and a 1.5-mm depth.[14,31] The manufacturer suggests this system should be used at 800 rpm with irrigating solutions after root canal preparation to size #25, allowing the instrument to reach difficult areas and even curved canals. XP-Endo Finisher has also the ability to remove intracanal medication[32,33,34] and biofilm[16] as well as to avoid apical extrusion of sodium hypochlorite,[35] including its effectiveness in curved root canals.[36] However, to date, only a few studies have evaluated the reduction of bacterial load by these instruments.[16,35]

The association of XP-Endo Finisher with XP-Endo Shaper or Reciproc Blue increased the effectiveness of CMP with the use of NaCl or NaOCl. Therefore, the null hypothesis was rejected. This finding is in line with Bao et al.,[16] who observed that XP-Endo Finisher associated with passive ultrasonic activation and conventional irrigation improved biofilm removal in infected root canals. This is also corroborated by Azim et al.,[35] who reported that XP-Endo Finisher presented higher bacterial reduction within the main root canal and better results than conventional irrigation.

Our results show that although bacterial load decreased, no instrumentation technique or tested irrigant was able to completely eliminate bacteria in oval-shaped root canals. Therefore, the development of new instrumentation/irrigation systems, including instrument design, biofilm-targeting irrigants, and substances capable of promoting better disinfection and enhancing host response, is necessary to increase the predictability of endodontic therapy.

Conclusion

In conclusion, our findings show that single-file instrumentation carried out with XP-Endo Shaper and Reciproc Blue files is effective in reducing

bacterial levels in oval-shaped root canals. The use of XP-Endo Finisher as a supplementary approach to the irrigation/instrumentation technique improved the cleaning efficiency of both file systems tested. Mechanical preparation performed with saline solution decreased culturable bacteria in the root canal, but antimicrobial substances such as NaOCl should be used to achieve a significantly better disinfection.

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