**Ex vivo ability of a noninstrumentation technique to disinfect oval-shaped canals**

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**Abstract**

**Context:** Oval-shaped canals represent a challenge in endodontics. Infected tissue may remain in their recesses. This concern may be more critical with minimally instrumentation techniques.

**Aims:** The present study evaluated the disinfection ability in oval-shaped canals of a noninstrumentation technique using ultrasonic agitation and intracanal heating of sodium hypochlorite (NaOCl) compared to rotary canal preparation and ultrasonic agitation with and without heating of NaOCl.

**Settings and Designs:** Sixty extracted mandibular incisors were included. The teeth had pulp necrosis and apical periodontitis and oval-shaped canals. They were divided into three groups depending on the treatment protocol: (1) IHAN: intracanal heating and ultrasonic agitation of NaOCl only, (2) R-IHAN: Rotary preparation followed by IHAN, and (3) R-passive ultrasonic agitation (PUA): Rotary preparation and ultrasonic agitation of NaOCl.

**Methods:** Root canal samples were taken before (S1) and after (S2) the endodontic procedures were completed and cultured anaerobically.

**Statistical Analysis Used:** Wilcoxon tests were performed to compare colony-forming units (CFUs) before and after the endodontic procedures for the three groups. The percentage of variation of CFUs was compared among the three groups using Kruskal–Wallis tests, followed by Mann–Whitney U-tests.

**Results:** All S1 samples were positive. All S2 samples showed bacterial growth in R-PUA compared to 17 in R-IHAN. None of the S2 samples in IHAN were positive. Bacteria reduction was significant in each group ($P < 0.001$). The percentage of bacteria reduction was highest for IHAN and lowest for R-PUA ($P < 0.001$).

**Conclusions:** Intracanal heating and ultrasonic agitation of NaOCl without instrumentation completely eliminated bacteria from infected oval-shaped canals.

**Keywords:** Disinfection ability; noninstrumentation technique; oval-shaped canals

**INTRODUCTION**

Apical disease is mainly caused by the presence of bacteria in the root canal system. Therefore, one of the objectives of a root canal treatment is the removal of bacteria. This is achieved by enlarging the canals to allow an effective irrigation with disinfecting solutions.

Different irrigation protocols have been proposed to aid in the killing of bacteria. Intracanal heating and ultrasonic agitation of sodium hypochlorite (NaOCl) following conventional root canal preparation has been reported to enhance canal cleanliness and disinfection in comparison to passive ultrasonic or sonic activation.

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**How to cite this article:** Yared G, Ramli GA. *Ex vivo ability of a noninstrumentation technique to disinfect oval-shaped canals.* J Conserv Dent 2020;23:10-4.
Preservation of tooth structure during an endodontic procedure is crucial for the long-term retention of the tooth.\textsuperscript{[8]} Minimally instrumentation techniques with laser-or ultrasonic-based irrigation techniques have been introduced with the aim to clean and disinfect the canal while preserving root structure.\textsuperscript{[9–11]}

To the present date, there is no information about the antibacterial ability of IHAN in canals that are minimally instrumented or un-instrumented. The present study evaluated the ex vivo antibacterial ability of a noninstrumentation technique using IHAN alone in comparison with a conventional rotary preparation followed by IHAN and a conventional rotary preparation with intracanal heating of NaOCl.

**METHODS**

Sixty mandibular incisors with pulp necrosis and asymptomatic apical periodontitis as confirmed by cold pulp testing and radiographs and freshly extracted for reasons not related to this study were selected. The following inclusion criteria were used: starting the protocol immediately after extraction, the lesion remained attached to the apex of the root,\textsuperscript{[12]} fully formed roots, absence of canal calcifications, absence of root fracture and resorption, pulp necrosis confirmed after access cavity preparation, presence of root canal infection confirmed by sampling and culturing, and the ability to place a #15 hand file to working length (WL) (detail below) and single oval-shaped canal at 3 mm short of the apex. The oval shape was determined from buccolingual and proximal radiographic projections. To be considered oval shaped, the buccolingual width of the canal had to be at least twice as large as the mesiodistal width.\textsuperscript{[13]}

Immediately following the extraction of the tooth, the crown was reduced to standardize the length of the tooth to 16 mm. The tooth was mounted and held in a vise. All procedures were done under strict aseptic conditions and a dental operating microscope at a \times 4 magnification. Rubber dam isolation was done. The crown of the tooth, the clamp, and the rubber dam were disinfected with 6% hydrogen peroxide followed by 5.25% NaOCl which was then neutralized with 10% sodium thiosulfate. The crown was reduced to standardize the length of the teeth to 16 mm approximately. The access cavity was prepared with sterile burs under sterile saline irrigation. The whole field was disinfected again with hydrogen 6% peroxide and 5.25% NaOCl followed by 10% thiosulfate to inactivate NaOCl. Sterility control samples were taken by rubbing sterile paper points (Reciproc paper points, VDW, Munich, Germany) against the cavosurface angle of the access cavity. Teeth were excluded if the sterility control samples were positive in an endpoint polymerase chain reaction using universal bacterial primers.\textsuperscript{[14,15]}

Intracanal samples were taken immediately before starting the intracanal procedures (sample S1) as described by Siddique et al.\textsuperscript{[15]} Sodium thiosulfate was placed in the access cavity and a size 15 hand file (Maillefer, Ballaigues, Switzerland) was used to carry the solution to WL established at 15 mm and to gently file the canal walls to suspend the canal contents in the thiosulfate solution. Sterile paper points were consecutively placed at WL to soak up the fluid. Each paper point was left in the canal for 1 min and then immediately placed into a sterile tube containing 1 mL Viability Medium Göteborg Agar III transport medium for microbiologic analysis. The transport media containing the root canal samples were thoroughly shaken for 60 s (Vortex; Marconi, Piracicaba, SP, Brazil). Serial 10-fold dilutions were made up to 10 − 3. Fifty microliters of the serial dilutions were plated onto 5% defibrinated sheep blood fastidious anaerobe agar (FAA; Lab M, Bury, UK) using sterile plastic spreaders to culture nonselective obligate anaerobes and facultative anaerobes. The plates were incubated at 37°C in an anaerobic atmosphere for up to 14 days. After this period, colony-forming units (CFUs) were visually quantified for each plate.

The tooth was excluded from the study if the hand file size 15 was unable to reach WL passively or if the sample S1 was negative.

The teeth were distributed into three groups: (1) Group R-passive ultrasonic agitation (PUA) (Rotary instrumentation and PUA) \((n = 20)\): the canal was prepared to the WL with BioRaCe rotary instruments from BR0 (25/08) to BR5 (40/04) driven by a VDW Silver motor, at 500 rpm and 1.0 Ncm according to the manufacturer’s instructions. Patency was checked with a #10 file and the canal irrigated with 3 mL of 5.25% NaOCl at room temperature each time the instrument was removed out of the canal and before using the next instrument. Approximately, 27 mL of NaOCl was used until BR5 reached WL. A NaviTip 31 G endodontic irrigation needle with double side port (Ultradent Products Inc.). At the end of the preparation procedure, the canal was irrigated with 5 mL of 17% ethylenediaminetetraacetic acid (EDTA) kept in the canal for 3 min followed by 5 mL of saline. Finally, the canal was filled with a room temperature of 5.25% NaOCl. An ultrasonic tip with a noncutting end (Irisafe tip K20/21 mm; Acteon, Mt Laurel, NJ) mounted in a piezoelectric ultrasonic device (P5; Satelec Acteon, Merignac, France) was inserted to 1 mm less than the WL and activated at the power setting of 4 for 20 s. PUA was repeated three times. The canal was flushed with 3 mL room temperature 5.25% NaOCl after each activation cycle to refresh the solution. (2) Group R-IHAN (rotary instrumentation followed by intracanal heating and ultrasonic agitation) \((n = 20)\). The canal preparation and irrigation were the same as for Group R-PUA. At the end of the preparation procedure, the canal was irrigated with 5 mL of 17% EDTA kept in the canal for 3 min followed.
by 5 mL of saline. Then, PUA was performed three times as described for Group R-PUA. After each time, NaOCl was aspirated, the canal was filled again with room temperature 5.25% NaOCl which was heated in the canal for 10 s using a Touch’n Heat XF (size 0.30 mm and 0.04 mm/mm taper) electric heat carrier (Analytic Endodontics, Orange, CA), attached to a System B unit (Analytic Endodontics). The temperature was set at 150°C. The heat carrier was inserted to 1 mm short of the WL. The canal was flushed with 3 mL room temperature 5.25% NaOCl after each activation cycle to refresh the solution. During the heating procedure, the heat carrier was moved with small, in- and out-movements in the canal. Care was taken to avoid wedging the heat carrier in the canal. (3) Group IHAN (Intracanal Heating and ultrasonic Agitation only) (n = 20). The canal was not shaped. NaOCl was placed in the canal, and PUA followed by and intracanal heating was performed three times as described for Group R-IHAN. After the first PUA, the canal was irrigated with 5 mL of 17% EDTA kept in the canal for 3 min followed by 5 mL of saline. The ultrasonic tip was introduced into the un-instrumented canal until binding then withdrawn 3 mm prior to activation.

After the endodontic procedure was completed, NaOCl was inactivated with 5 mL of 5% sodium thiosulfate followed by saline. The root canal was sampled (sample S2) and CFUs were visually quantified as described for sample S1.

**Statistical analysis**

The Statistical Package for the Social Sciences (SPSS for Windows, Version 22.0, Chicago, IL, USA) Software was used to perform the statistical analysis. The level of significance was set at \( P \leq 0.05 \). Kolmogorov–Smirnov tests were conducted to evaluate the normality distribution of continuous variables. Wilcoxon tests were performed to compare CFUs before and after the endodontic procedures for the three groups. The percentage of variation of CFU after irrigation was compared among the three groups using Kruskal–Wallis tests, followed by Mann–Whitney U-tests.

**RESULTS**

The results are shown in Table 1.

The number of CFUs decreased significantly from S1 to S2 samples for the different groups \( (P < 0.001) \). The percentage of bacteria reduction was very high for Groups R-PUA and R-IHAN but significantly different from 100% \( (P < 0.05) \). It was higher for R-IHAN. All of the S2 samples were positive for R-PUA and 17 were free of bacteria for R-IHAN.

All S2 samples were free of bacteria in IHAN. The percentage of bacteria reduction was higher for IHAN compared to R-IHAN and R-PUA \( (P < 0.001) \).

**DISCUSSION**

The ex vivo effectiveness of IHAN as a final irrigation protocol following root canal preparation on the elimination of bacteria has been recently demonstrated. R-IHAN resulted in better root canal disinfection compared to intracanal heating and sonic or ultrasonic activation of NaOCl in canals infected with *Enterococcus faecalis* after extraction of the teeth. However, the experimental setup did not allow to replicate an actual root canal infection caused by multiple microorganisms forming a well-established biofilm in contrast to the present study in which infected teeth with apical periodontitis were used.

This was the first study to evaluate the disinfecting ability of a noninstrumentation technique. Conventional root canal sampling and culturing methods were used to assess the antibacterial ability in the present study. Alves et al. demonstrated that those techniques were as reliable as quantitative real-time polymerase chain reaction for this purpose. However, the results of the present study should be carefully interpreted due to the limitations of the conventional sampling and culturing technique.

Oval-shaped canals represent a challenge to cleaning and disinfection. The instruments are unable to reach the recessed areas leaving infected dentine and pulp tissues intact. In addition, debris is packed in the recessed areas. Oval-shaped canals were used in the present study because they would be suitable to assess the antibacterial ability of a noninstrumentation technique.

In accordance with previous studies on teeth with apical periodontitis, all of the S1 samples, before treatment, were positive.

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Table 1: Mean and standard deviation of colony-forming units in each group, for S1, S2 and the percentage of difference in colony-forming units between S2 and S1

| Irrigation protocols†† | S1 a,b,c | S2 a,b,c | Percentage of S2-S1‡ |
|------------------------|---------|---------|---------------------|
| R-PUA                  | 1.02 × 10±2.9 × 10³ | 9.94 × 10±1.8 × 10³ | 97.451±0.672 a       |
| R-IHAN                 | 9.76 × 10±4.2 × 10³ | 8.10 × 10±2.9 × 10³ | 99.917±0.063 b       |
| IHAN                   | 8.84 × 10±2.5 × 10³ | 0           | 100.00±0.00 c                 |

aSample taken before (S1) and after (S2) irrigation, bNumber of colony-forming units was significantly less after irrigation compared to before irrigation for the different groups \( (P<0.001) \), cPercentage of S2-S1: Percentage of bacteria reduction, Different superscript letters \( (a,b,c) \) indicate statistical difference. ††R-PUA: Rotary instrumentation followed by intracanal heating and ultrasonic agitation, R-IHAN: Rotary instrumentation followed by intracanal heating and ultrasonic agitation, PUA: Passive ultrasonic agitation, IHAN: Intracanal heating and ultrasonic agitation only
All three protocols were very effective in reducing bacterial counts. In agreement with a recent study, R-IHAN was significantly better than R-PUA.[7] This difference could be attributed to the increased ability of intracanal-heated NaOCl to kill bacteria and to dissolve pulpal tissue and a better penetration of NaOCl in dentinal tubules. None of the S2 samples in group R-PUA were free of bacteria. This finding confirmed that mechanical canal preparation followed by PUA could not consistently render the canals free of bacteria.[7,22,23] Seventeen S2 samples from R-IHAN were free of bacteria. In Yared et al., all S2 samples from R-IHAN were negative.[7] The difference in the results was related to the type of teeth/canals used. In the present study, oval-shaped canals in mandibular incisors were used compared to mandibular premolars in the study of Yared et al.[7] Another explanation was the type of infection as aforementioned.

The results of the present study were surprising: IHAN alone, without canal preparation, was also very effective in reducing the number of bacteria. More bacteria were killed with IHAN alone compared to the other two groups. Moreover, unexpectedly, all S2 samples did not show any bacterial growth despite the fact that a root canal preparation was not done. Although passive ultrasonic irrigation could form a smear layer,[24] it could be assumed that less smear layer and debris were formed in group IHAN (without rotary instrumentation) compared to group R-IHAN (with rotary instrumentation) allowing a better exposure of bacteria to the heated NaOCl, especially in the hard-to-reach recessed areas of an oval-shaped canal.

The high incidence of negative S2 samples (30/30) after IHAN alone was an interesting finding. The absence of intra-radicular bacteria prior to obturation was associated with a higher healing rate than if bacteria were present.[25-27]

Conventionally, elimination of intracanal bacteria was attributed to the association of canal shaping and irrigation on the basis that effective irrigation required adequate canal enlargement. The results of the present study showed that canal preparation was not a requisite to eliminate bacteria from infected oval-shaped canals in mandibular incisors.

**CONCLUSIONS**

One of the main objectives of a root canal treatment is to eliminate bacteria. This can be achieved through adequate canal preparation and irrigation with NaOCl. Ultrasonic activation of intracanal heated NaOCl as a final irrigation protocol following canal preparation demonstrated greater bacterial reduction than canal preparation and NaOCl activation alone. However, the antibacterial ability of this protocol has never been evaluated in canals that were not instrumented. The present study showed that canal preparation was not essential to eliminate bacteria from infected root canals. Ultrasonic agitation of 5.25% NaOCl heated in non-instrumented oval-shaped canals was more effective than the conventional rotary preparation with or without IHAN. The noninstrumentation technique with IHAN is promising with regard to the elimination of bacteria. Further evaluation is needed.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

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