Evaluation of the mono instrument (Wave One) mechanical action on the bacterial load reduction: In vitro study of 32 permanent human teeth

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Abstract

Context: Enterococcus faecalis is the most common bacteria found in infected root canals.

Aim: The purpose of this study was to evaluate the effect of the mono instrument (wave-one®) on the reduction of E. faecalis in root canals.

Materials and Methods: Thirty-two human monoroot teeth were used. After sterilization by autoclave, the teeth were infected by E. faecalis and incubated for 24 h. Each tooth underwent sampling before and after the root canal shaping. After serial dilution, samples were incubated, and colony-forming units were counted.

Results: The mono instrument technique reduced infection by E. faecalis in root canals of 30 teeth. The mean bacterial load (log_{10}) was 3.98 before treatment and 1.20 after treatment. The paired t-test showed a significant mean difference (log_{10}) of the bacterial load before and after treatment (P < 0.0001).

Conclusion: This study found that the mono instrument (Wave One®) significantly decreases bacterial load in root canals. However, the instrument alone is not enough to eradicate infections; thus, the use of a complementary antimicrobial is required.

Keywords: Antimicrobial activities; Enterococcus faecalis; root canal; Wave One®

INTRODUCTION

The role of bacteria and their metabolites in the pathogenesis of pulp and periapical pathology was demonstrated in 1965 by Kakehashi et al.[1] Antibacterial control is an important element in the success of endodontic treatment, which involves both mechanical and chemical means. Root canal shaping is a key step in endodontic treatment that when performed correctly, is a predictor of success.[2] However, it only applies to the main canal containing the majority of the bacterial flora and allows root canal irrigants to enter the secondary lateral and accessories root canals.[3] Studies showed that instrumentation reduces up to 90% of bacteria in the root canal without antibacterial irrigation.[4,5] This aim is hard to achieve using stainless steel manual instruments.[6] The introduction of continuous rotation with nickel–titanium (NiTi) instruments was an important step in optimizing shaping of root canals.[7] This approach is quicker, safer, and more reproducible with a small risk of error in the procedure when compared with manual instrumentation.[8] These NiTi instruments are flexible, have increased cutting efficacy, maintain the original shape of the root canal during preparation, and have a reduced tendency to transport the apical foramen. The current trend is to use a mono instrumentation with the aim of

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simplifying protocols and root canal shaping time while maintaining and improving results.

Thus, WaveOne® introduces a unique shaping system with reciprocity motions that can be used in any situation, regardless of the length, the diameter, and the curvature of the root canal. Based on the literature review on root canal shaping using WaveOne®, researches are more oriented toward time saving and reducing instrument fracture and periapical debris extrusion. Few studies explore the technique’s ability to reduce the number of microorganisms in infected teeth’s root canals.

The aim of our study was to evaluate in vitro reduction of bacterial load by mechanical action of a mono instrument sequence, Wave One®, on permanent human teeth contaminated with strains of Enterococcus faecalis.

**MATERIALS AND METHODS**

We conducted an experimental study at the Department of Odontology of the Faculty of Medicine and the Microbiological Research Unit of the Hospital.

Thirty-two mature human permanent monoradicular teeth without decay, crack, or fracture, external or internal resorption, calcification, or previous root canal treatment extracted for periodontal or orthodontic reasons were selected. Teeth were disinfected in a 2.5% solution of sodium hypochlorite for 1 h, rinsed in physiological saline, and then dried.

Glass ionomer cement Fuji II LC (GC®, America, Inc.) was placed at the apex of selected teeth. The external aspect of roots was covered by a layer of varnish. Teeth were individually mounted on plaster blocks to facilitate their manipulation and identification.

Conventional endodontic access was performed using a Tungsten carbide drill #8 or #12 (Dentsply Maillefer, Swiss) mounted on an air turbine. An Endo Z drill (152 Dentsply Maillefer, Swiss) was used to remove the pulp chamber’s roof.

X-rays, with #10 K-files (Dentsply Maillefer, Swiss), placed in root canals, including retro-alveolar films (Kodak®, Paris, France) and a long cone X-ray tube (Prodental®), were performed to determine the canal working length for each tooth. Teeth were then individually conditioned under a vapor bag (REXAM SPS, Coulommiers, France) and sterilized by autoclave at 121°C for 20 min. The same operator conducted all procedures.

Two teeth were randomly selected and placed individually in a sterile disposable bottle of polyethylene to verify the absence of germs after sterilization. The root canal was filled with physiological saline and the bottle was incubated at 37°C for 24 h. After incubation, they were filled again with physiological saline and pumping movements were performed by a #10 K-files.

A sample of 10 μl was withdrawn from each root canal and diluted in 1 ml of physiological saline. The resulting solution was inoculated by spreading it in two Petri dishes which were incubated for 24 h at 37°C. The absence of bacterial growth after 24 h indicated the absence of germs.

**Preparation of the inoculum**

The inoculum was prepared with the reference strain, E. faecalis ATCC 29212 (Lustiner, France), from a 24 h pure culture obtained on agar from fresh blood. Wells were identified and perfectly identical colonies were scraped by a Platin hook and discharged in 5 ml of 0.9% sterile physiological saline. Using a densitometer, the suspension of E. faecalis was well homogenized and adjusted to get an opacity equivalent to 0.5 Mc Farland, which has a bacterial density of 3.5.10⁶ cfu/ml.

**Teeth contamination**

Bags containing teeth were opened under an oven with laminar air flow and the root canal of each tooth was contaminated with the inoculum. After filling the root canal with the inoculum of E. faecalis, pumping movements by #10 K-files spread the suspension through the length of the root canal. The inoculated teeth were individually placed in a single-use sterilized polyethylene bottle and placed in an oven at 37°C for 24 h.

**Sampling and counting before root canal preparation**

A first sample (P1) was collected 24 h after teeth contamination. The teeth were removed from the oven and dried. Each root canal was filled again with physiological saline; pumping movements by #10 K-files spread the physiological saline through the length of the root canal and put the bacterial content in suspension. 10 μl was collected and put in a tube containing 5 ml of physiological saline and homogenized by shaking the tube.

A series of dilutions to 1/10, 1/100, and 1/1000 was performed and 0.1 ml of each dilution was seeded in Petri dishes containing agar and fresh blood and incubated at 37°C for 24 h. After incubation, bacterial count was performed using a colony counter to determine the number of colonies forming units per ml (cfu/ml) [Figure 1].

**Root canal preparation**

The root canal was explored by a #10 K-file, irrigated by physiological saline, and enlarged by a WaveOne® Primary 25/100, which followed the penetration length of the #10
The WaveOne® was used with slight movements in a back-and-forth manner progressing toward the apex. After 2 or 3 back and forth movements or a feeling of blockage, the WaveOne® was removed and cleaned with sterile gauze and the root canal irrigated with physiological saline. The instrument was reintroduced in the root canal and moved more toward the apex without pressure.

This cycle was repeated until the instrument reached 1 mm from the apical foramen.

Irrigation during instrumentation was performed with a total of 5 ml of physiological saline for each tooth using a plastic syringe with a needle of 16 mm length and 50/100 mm diameter. All teeth were prepared under a sterile hood.

**Sampling and counting after root canal preparation**

A second sample (P2) was drawn immediately after root canal preparation following the same process as for the first sample. Bacterial counting was performed after the serial dilution [Figure 2].

This second sample was compared to the first sample to evaluate the efficacy of the instrument.

**Data management and statistical analysis**

Data of each tooth were entered in Excel spreadsheet (Microsoft 2013 version).

Results of bacterial count in cfu/ml were transformed in Log10 before statistical analysis in Epi Info®.

The percentage of bacterial count reduction after treatment by WaveOne® was determined. The effect of WaveOne® on the bacterial load was evaluated by comparing the number of bacteria in each root canal before and after root canal shaping. A paired Student’s t-test was used to compare the median of bacterial loads before and after treatment. The differences were statistically significant if $P < 0.05$.

## RESULTS

Cultures of samples from two controlled teeth were negative after 24 h of incubation. The sample of the thirty infected teeth had a positive culture with a mean bacterial load of 23,440 cfu/ml (3.98 in log10).

WaveOne® mono instrument technic reduced the quantity of *E. faecalis* in root canals of thirty teeth.

The mean bacterial load (log10) before treatment was 3.98 compared to 1.20 after treatment.

Paired $t$-test showed a significant difference in the mean (log10) bacterial loads before and after treatment with a $P < 0.0001$. The percentage of mean reduction of bacteria quantity was 69.8% [Table 1].

## DISCUSSION

One of the most important objectives of canal instrumentation is to eliminate vital or necrotic pulp, infected dentin, and dentin debris. This will eradicate most microorganisms in root canals. The current laboratory study evaluated the efficacy of WaveOne® in the reduction of canal bacterial load by mechanical action alone.

### Table 1: Mean bacteria population before and after treatment

| Teeth | Mean before treatment (cfu/ml) | Mean before treatment (Log10) | Mean after treatment (cfu/ml) | Mean after treatment (Log10) | Mean reduction (%) |
|-------|-------------------------------|------------------------------|----------------------------|------------------------------|-------------------|
| 30    | 23440±5480                    | 3.98±0.11                    | 49.4±16                     | 1.20±0.12                   | 69.8              |

**Figure 1**: Counting before root canal preparation

**Figure 2**: Counting after root canal preparation
Primary endodontic infections are always characterized by the presence of several bacterial species forming, an ecosystem that is very labile over time making it impossible to reproduce in vitro. That is why E. faecalis was used instead of arbitrarily using a diversity of germs that will never be representative of a real endocanal bacterial environment. E. faecalis is the most commonly used microorganism because of its ability to colonize dentin tubuli. This choice can also be justified by the fact that this germ is the cause of secondary and persisting infections due to its capability to colonize the root canal to its most inaccessible areas. Furthermore, E. faecalis is very frequently present in endodontic infections. Peciuliene et al. studied microbiological status of forty teeth with periradicular lesions and found Enterococcus in 64% of cases. In a similar study, Pinheiro et al. reported that E. faecalis was the most frequently recovered bacterial species and the microbial flora within root canals of teeth with failed root canal treatment. Chemomechanical procedures cannot neutralize it, causing treatment failure. E. faecalis was regularly used in different in vitro studies of canal disinfection, especially since its optional anaerobic feature allows its manipulation and culture.

The incubation period was 24 h in our study. This period was based on Hubble et al.'s findings which indicated that in 24 h, microorganisms were present in the whole canal system and could be found in the dentinal tubuli at up to 300 μm depth. These findings are in accordance with results of Haapasalo and Orstavik’s study indicating that E. faecalis is found at a depth of 300 to 400 in bovin dentinal tubuli after 24 h incubation at 37°C. However, Siqueira et al. used an incubation time of 48 h to increase the depth of bacterial invasion into the dentinal tubuli.

Indeed, because the study focused on Wave One® mechanical action, no antimicrobial irrigation was used. A chemical disinfectant could interfere with the role of the instrument in eliminating microorganism in the root canal. The physiological saline used had neither antimicrobial action, nor a flow effect on bacteria and could serve as a culture medium for E. faecalis.

The WaveOne® instrument was effective in significantly reducing the number of microorganisms in the canal lumen and dentin walls at up to 69.8% (P < 0.0001).

This result is corroborated by Nabeshima et al.’s findings, showing that WaveOne® and OneShape® significantly reduced the number of bacteria in the root canal.

Karataş et al. also found that WaveOne® was significantly effective in the reduction of E. faecalis in root canals. That reduction could be explained by alternative instrument movements, the distribution of blades, a variable angle, and an helicoidal step that increases the flexibility, allowing an increased cutting efficacy and a better evacuation of debris. WaveOne® instrument has an inverted screw and two distinct transversal sections in its length with a transversal triangular modified convex section. These features allow counterclockwise cuts in a more significant way as opposed to the clockwise fashion. This facilitates the progression of the instrument toward the apex; the progressive liberation of constraints opposing the instrument during the preparation and maintains the instrument centered while preventing a screwing and moreover eliminates the infected dentin from canal walls. Similar results were published by Goldberg et al., back-and-forth movements yield a greater cutting action compared to the disengagement, a better progression toward the apex, and more efficacy.

With the advent of new technologies, other studies could be conducted with more instruments and canal preparation technics, including laser, which is more and more used in endodontic procedures. However, the complex root canal anatomy limits mechanical actions of endodontic instruments. Therefore, a maximal disinfection of root canals requires completing the mechanical preparation by chemical solutions that have antimicrobial actions, lubricant properties, and which are able to dissolve organic tissues with low toxicity.

CONCLUSION

This study indicates that the instrument used can significantly eliminate intracanal bacteria. However, combining mechanical preparation with a chemical disinfectant is necessary to optimize the eradication of bacteria in root canals.

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Conflicts of interest
There are no conflicts of interest.

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