The time dependent effect of various irrigation systems on the reduction of E. faecalis in experimentally infected root canals

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Objective: To compare the time dependent effect of Vibringe, EndoVac, non-activated SAF, passive ultrasonic irrigation (PUI), and a conventional syringe on the reduction of E. faecalis in experimentally infected root canals.

Methods: Sixty human mandibular premolar teeth with straight roots and single root canals, were infected with E. faecalis. One group served as a control group and in the control group, neither irrigation nor instrumentation procedures were performed. The root canals were irrigated with Vibringe, EndoVac, non-activated SAF, passive ultrasonic irrigation (PUI) and conventional syringe using distilled water. After the irrigation procedures, samples were taken from root canals with paper points and incubated in blood agar plates. The colonies grown on the blood agar were counted and interpreted as colony forming units per millilitre.

Results: Intergroup analysis revealed significant differences between the control and experimental groups (p<0.001). The root canal irrigation with the conventional needle for four minutes was more effective than two minutes at reducing the number of cfu counts.

Conclusion: It can be concluded that the irrigation time with the conventional needle significantly affects the reduction of E. faecalis in the root canal.

Keywords: EndoVac; SAF; time-dependent; vibringe.

The presence of bacteria in the root canal system is the primary etiological factor in the development of pulp and periapical diseases.[1–3] One of the main objectives of endodontic treatment is the complete elimination of intracanal bacteria or at least to reduce them to the levels that are compatible with periapical tissue healing.[4]

Although mechanical preparation of the infected root canals is particularly effective in reducing the presence of bacteria in the main root canal, it is not reliable in rendering canals bacteria free.[5,6] Studies have shown that the use of irrigants and medicaments provides an additional antibacterial effect in combination with mechanical preparation.[7–9] However, the incidence of negative cultures ranges from 40%–60% of the cases after chemo-mechanical preparation with different instrumentation techniques and instruments, and conventional irrigation with different irrigants.[10–16] In this regard, to improve root canal disinfection, various irrigation systems were introduced in endodontics.
The Self-Adjusting File (SAF) (ReDent Nova, Ra’anana, Israel) is a hollow and flexible instrument that removes dentine with a back-and-forth grinding motion. An advantage of the SAF system is that the irrigant is continuously delivered throughout instrument operation by a peristaltic pump. It can also be used as a non-activated device for the irrigation of root canals without activating the file.[17,18]

The Vibringe System (Vibringe B. V. Corp, Amsterdam, Netherlands) is an irrigation device that allows continuous sonic irrigation of the canal system during endodontic treatment. However, the effects of the Vibringe and non-activated SAF on reduction of Enterococcus faecalis (E. faecalis) in experimentally infected root canals have not been reported.

The irrigation time also has an effect on the disinfection of experimentally infected root canals. Although there are studies that have evaluated the efficacy of irrigation solutions and their optimal concentration, contact time and temperature for clinical use,[19–21] a study that evaluates the effect of irrigation systems and their optimal usage time on the reduction of E. faecalis in experimentally infected root canals without using any antibacterial irrigation solution has not been reported. Therefore, the aim of the present study was to compare the time dependent effect of Vibringe, EndoVac (Discus Dental, Smart Endodontics, Culver City, CA, USA), non-activated SAF, passive ultrasonic irrigation (PUI) (NSK, Nakanishi Inc. Kanuma, Japan), and a conventional syringe on the reduction of E. faecalis in experimentally infected root canals. The null hypothesis is that there is no difference between these systems and irrigation time on eliminating E. faecalis from experimentally infected root canals.

**Materials and methods**

Sixty human mandibular premolar teeth with straight roots and single root canals, extracted for reasons not related to this study, were selected. All teeth had complete root development and the presence of a single canal was established by radiographs taken in both mesiodistal and buccolingual projections. Only those teeth with root canals presenting a >2.5:1 ratio between the buccolingual and mesiodistal dimensions at a level 5 mm from the apex were included in the experiment. The crowns were sectioned with a high-speed burr (KG Sorensen, Barueri, SP, Brazil) under a copious water spray and the root lengths were standardized to approximately 14 mm. The working length (WL) was obtained by measuring the length of the initial instrument (size 15) at the apical foramen minus 1 mm. Root canals were instrumented to a size 20 K-file (Mani Inc. Tochigi, Japan) under copious irrigation with distilled water. Composite resin was used to seal the apex and the root surfaces were covered with nail varnish to prevent the leakage of bacteria. To simplify the manipulation during contamination and irrigation procedures, specimens were mounted vertically up to the cemento-enamel junction in blocks made of acrylic resin. All samples were placed in autoclave sachets and sterilized in autoclave at 121 °C for 20 minutes. The effectiveness of the sterilization was checked with an indicator placed in the sachets.

The E. faecalis strain (ATCC 29212) was used to contaminate the root canals. A microbial suspension containing approximately 10⁶ colony forming units (cfu)/mL was prepared by mixing a pure culture of E. faecalis and sterile phosphate-buffered saline. The density of 0.5 McFarland was measured by the densitometer (BioMérieux, Lyons, France). The canals were contaminated with 20 µL of the suspension containing E. faecalis, using automatic micropipets, and size 15 K-files were used to carry the suspension to the WL. The acrylic blocks containing teeth were then incubated at 37 °C for 48 hours.

After contamination, all teeth were randomly divided into six groups: control, needle, non-activated SAF, Vibringe, Endo Vac, and PUI.

In all the groups, except the control group, the root canals were instrumented using Protaper Universal rotary files (Dentsply Maillefer, Ballaigues, Switzerland) to a size of 40, .06 taper. The following sequence was used: F2, F3 and F4 files (full WL).

In the control group, neither irrigation nor instrumentation procedures were performed.

In the needle group, the irrigation was performed with a syringe and a 30 gauge (approximately 0.25 mm) closed-end tip and side-port opening needle (Canal Clean, Biodent, South Korea). The root canals were irrigated with the needle for one minute using 1 mL of distilled water between each file. Following the use of the last instrument, the needle was used for two minutes using 2 mL of distilled water as a final rinse. The first sample was taken from the root canal as described later. The irrigation procedure was performed for two more minutes and a second sample was taken. The flow rate of the irrigating solution was 1 mL min⁻¹. A new needle was used for all teeth to prevent the root canals from cross contamination.

In the non-activated SAF group, the SAF system was used only for irrigation with a VATEA peristaltic pump as described previously.[18] A 1.5 mm diameter and 25 mm length SAF file was operated for one minute at a rate of 1 mL/minute between each file. After the last instrument, the SAF system was used for two minutes as a final rinse and the first sample was taken from the root canal.
The SAF system was used again for two more minutes at the same flowing rate and a second sample was taken from the root canal. A new SAF instrument was used for all teeth to prevent the root canals from cross contamination.

In the Vibringe group, the root canals were irrigated and sonically activated via the Vibringe system (Vibringe B. V. Corp, Amsterdam, Netherlands) at a 1 mL/minute flowing rate with the same irrigation needle used for the needle group. The Vibringe system was used for one minute between each file and, after the last instrument, it was used for two minutes as a final rinse and the first sample was taken from the root canal. For the second sample, the Vibringe system was used for two more minutes at the same flowing rate. A new needle was used for all teeth to prevent the root canals from cross contamination.

In the EndoVac group, the master delivery tip of the EndoVac system was used for irrigation at a 1 mL/minute flowing rate and the solution was evacuated via the microcannula. After every six seconds, the microcannula was withdrawn 2 mm for 6 seconds to evacuate micro bubbles and insure a constant irrigant exchange. Then, the microcannula was used 1 mm short of the WL. The root canals were irrigated with the EndoVac for one minute between each file, and after the last instrument, it was used for two minutes as a final rinse. The first sample was then taken from the root canal. The second sample was collected after using the Vibringe system was for two more minutes at the same flowing rate. A new microcannula was used for all teeth to prevent the root canals from cross contamination.

In the PUI group, the root canals were irrigated with the same irrigation needle used for the needle group at a 1 mL/minute flowing rate between each file. After each irrigation procedure, a U-file ultrasonic tip (size 15, 0.02 taper) (NSK Varios; Nakanishi Inc., Tochigi, Japan) was placed 1 mm short of the WL and activated at a frequency cycle of 28 kHz-32 kHz per second for one minute. Following the use of the last instrument, the root canals were irrigated for two minutes as a final rinse. The ultrasonic tip was activated at the same frequency for two minutes and the first sample was taken from the root canal. For the second sample, the root canals were irrigated and the solution was activated for two more minutes at a 1 mL/minute flowing rate and a frequency cycle of 28 kHz-32 kHz per second, respectively. A new ultrasonic tip and needle was used for all teeth to prevent the root canals from cross contamination.

In all groups, a total of 5 mL of distilled water was used for irrigation and the needle tip was placed 1 mm short of the WL in needle, Vibringe, and PUI groups.

After the irrigation procedures, three paper points were placed into the root canal at WL and each paper point remained in the root canal for one minute. Paper points were transferred into tubes containing 1 mL of 0.85% sterile saline and then vortexed thoroughly for one minute. After 10 fold serial dilutions in sterile saline, aliquots of 0.1 mL were plated onto blood agar plates and incubated at 37 °C for 24 hours. The colonies grown on the blood agar were counted and interpreted as colony forming units per millilitre (cfu/mL).

The data were analyzed using the two-way analysis of variance (ANOVA) and Tukey’s post hoc tests in order to detect the effect of the independent variables (groups and time), and their interactions on the reduction of the cfu counts (p<0.05). All statistical analyses were realized by using software (SigmaStat for Windows Version 3.5; Systat Software, Inc., Erkrath, Germany) at a significance level of 0.05 and a confidence interval of 95%.

### Results

The two-way ANOVA indicated that the cfu count was significantly affected by the groups (p<0.001), and by the time (p<0.05). However, there were no statistically significant interactions between the groups and time (p=0.605) (Table 1).

The number of cfu counts of E. faecalis after irrigation with the conventional needle, nonactivated SAF, Vibringe, EndoVac, and PUI for two and four minutes is presented in Fig 1. Intergroup analysis revealed significant differences between the control and experimental groups. All irrigation systems were significantly effective when compared with the control group (p<0.001). However, there were no statistically significant differences between experimental groups (p>0.05).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>5</td>
<td>87813549.074</td>
<td>17562709.815</td>
<td>282.042</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time</td>
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<td>253267.593</td>
<td>253267.593</td>
<td>4.067</td>
<td>0.047</td>
</tr>
<tr>
<td>Group × Time</td>
<td>5</td>
<td>226215.741</td>
<td>45243.148</td>
<td>0.727</td>
<td>0.605</td>
</tr>
<tr>
<td>Residual</td>
<td>96</td>
<td>5977911.111</td>
<td>62269.907</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>107</td>
<td>94270943.519</td>
<td>881036.855</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DF: Degrees of freedom; SS: Sum of squares; MS: Mean squares
Intragroup analyses revealed that the root canal irrigation for two and four minutes yielded a significant bacterial reduction. In the conventional needle group, a significant difference was observed when comparing two and four minutes (p<0.05). The root canal irrigation with the conventional needle for four minutes was more effective than two minutes at reducing the number of cfu counts. There were no significant differences when comparing two minute and four minute irrigations with nonactivated SAF, Vibringe, EndoVac, and PUI groups in terms of bacterial reduction in the root canals.

**Discussion**

The foundation of clinical endodontics is the microbial control and the prognosis of endodontic treatment depends on root canal disinfection. Du et al. indicated that the killing of bacteria in infected dentin by root canal disinfecting solutions is time-dependent. Although there are many studies that have evaluated the efficacy of different irrigation techniques on root canal disinfection, the time-dependent effect of Vibringe, EndoVac, non-activated SAF, PUI, and the conventional syringe on the reduction of E. faecalis in experimentally infected root canals has not been studied. Therefore, in the current investigation, the time-dependent ability of five different irrigation techniques in reducing E. faecalis from root canals during chemomechanical instrumentation was evaluated.

The results of the present study demonstrated that all techniques provided a significant reduction in the bacterial populations when compared with the control group and there were no statistically significant differences among the experimental groups. Previously, the ability of non-activated SAF irrigation in reducing E. faecalis has not been studied. Therefore, a direct comparison cannot be done with studies in which an activated SAF was used. Further studies should be conducted to confirm the findings of the present study.

In the present study, there was no significant difference between the nonactivated SAF irrigation for two or four minutes. This finding is in accordance with the result of a previous study which evaluated the time-dependent antibacterial effect of the SAF and found that there was no significant difference when comparing the SAF instrumentation for two minutes with four minutes.

The EndoVac system was claimed to be able to increase cleaning and disinfection of the root canal. Several studies found better results with the EndoVac system than with conventional or ultrasonic irrigation in terms of the cleaning of the root canal. On the other hand, Brito et al. compared the effectiveness of three irrigation systems in reducing intracanal E. faecalis populations and concluded that there was no significant difference between the EndoVac and other irrigation techniques. Miranda et al. also compared the antimicrobial efficacy of the conventional needle irrigation, EndoVac system and photodynamic therapy against intracanal E. faecalis and found that there was no significant difference among the the groups. In the present study, the EndoVac system provided similar results to those of other irrigation techniques. The critical methodological differences in the experimental models, such as the volume and type of irrigant, may explain such divergences amongst the studies that evaluated the antimicrobial efficacy of the EndoVac system.

The direct contact of the root canal irrigation solution with the bacteria in peripheral areas of the root canal is often impossible. Caron reported that the sonic activation of the irrigant provides deeper penetration of the irrigant to all areas of the endodontic space. Although previous studies have demonstrated that sonic activation of the irrigant is more effective in reducing bacterial load in the root canal, one study reported that sonic activation of the irrigant is not better than needle irrigation at removing bacteria from the root canals.

The possible influence of the size of the sonic or ultrasonic oscillating tip upon the irrigation has been stated. The Vibringe system allows sonic oscillation of the needle via its specially designed disposable syringe, which is compatible with every irrigation needle. The differences between the size of the oscillating tip, type and amount of irrigant might have led to different results.

The use of ultrasonic to increase debridement and disinfection has been suggested. However, in the present study, there was no significant difference between the PUI and other experimental groups, which is in accordance with a previous study that compared the PUI with needle irrigation.

The result of the present study showed that the root canal irrigation with the conventional needle for four minutes is more effective than two minutes in reducing
the number of cfu counts. A recent study compared the effect of long-term exposure to endodontic disinfecting solutions on young and old *E. faecalis* biofilms in dentin canals and found that the proportion of killed bacteria was significantly lower after three minutes than after 10 and 30 minutes of exposure to the disinfecting agents. In the present study, an antibacterial irrigation solution was not used. The increased effect in four minute groups might be due to the volume of the irrigant, which has a mechanical influence on bacterial elimination.[42,43]

*E. faecalis* was selected for the study because it is known to be one of the most resilient bacteria and is associated with both primary and persistent endodontic infection, it can penetrate deep into dentinal tubules to form communities organized in biofilms and it has the ability to survive under unusual environmental stress.[44–48]

Since our aim was to assess the effect of the irrigation techniques in reducing *E. faecalis* from root canals, distilled water, which exerts no antibacterial effect on *E. faecalis*, was used as an irrigant in this study.[49]

**Conclusions**

In conclusion, the findings of this study revealed that all irrigation techniques tested were effective on the reduction of *E. faecalis* in the root canals. The root canal irrigation with the conventional needle reduced more cfu counts in four minutes than it did in two minutes. Therefore, it can be concluded that the irrigation time with the conventional needle significantly affects the reduction of the bacterial count in the root canal. However, there were no differences between two and four minutes of irrigation with the nonactivated SAF, VibeRinge, EndoVac and PUI groups in reducing *E. faecalis* counts from the root canals.

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**References**

Karataş et al. *E. faecalis* in experimentally infected root canals

