Antibacterial efficacy of endodontic irrigating solutions and their combinations in root canals contaminated with Enterococcus faecalis

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Objective. The objective of this study was to evaluate the antibacterial efficacy of irrigating solutions and their combinations against Enterococcus faecalis.

Study design. One hundred ten single-rooted human teeth were inoculated with E. faecalis and incubated for 21 days. Teeth were divided according to the irrigant: Group I (GI), 2.5% sodium hypochlorite solution (NaOCl); GII, 2.5% NaOCl/H11001 10% citric acid; GIII, 2.5% NaOCl/apple cider vinegar; GIV, apple cider vinegar; GV, 2% chlorhexidine solution; GVI, 1% peracetic acid; GVII, saline solution. Microbiological samples were taken after root canal preparation and 7 days later. Data were submitted to ANOVA (5%).

Results. All solutions promoted reduction of E. faecalis after instrumentation, but bacterial counts were higher in the final sample. GI, GV, and GVI had lower bacterial counts than the other groups.

Conclusions. The irrigating solutions may present activity but do not eradicate E. faecalis in the root canal system.

Microorganisms and their products play an essential role in the development of pulpal and periapical pathologies.1-3 Culture and molecular techniques allowed the detection of more than 460 species of bacteria in different types of endodontic infections.4,5 Enterococcus faecalis is a gram-positive facultative anaerobe capable of invading the dentin tubules6 and is resistant to several irrigating solutions and medications used in endodontics.7

Sodium hypochlorite (NaOCl) is the most commonly used irrigating agent because of its broad microbicidal activity and tissue-dissolving capability.8,9 However, variables, such as concentration,10 temperature,11 and pH,12,13 can affect its efficacy. At lower pH, the chlorine available in the NaOCl solution presents as hypochlorous acid (HOCl), which is more active than the hypochlorite anion (OCl−) that prevails at higher pH.14,15 Therefore, the combination of NaOCl with acidic solutions, despite affecting its stability, may increase its antimicrobial effect.12

Chlorhexidine is a potent antiseptic, used as an endodontic irrigant, presenting a wide spectrum of antimicrobial action and substantivity.16-18 Its main disadvantage is the lack of ability to dissolve organic matter,19 which negatively affects its cleaning capacity.20

Alternative irrigating solutions demonstrating antibacterial action and ability to clean the dentin walls have been investigated. Apple cider vinegar is active against endodontic microorganisms21 and has the ability to remove the smear layer.22 Peracetic acid is a disinfectant widely used in the food industry and in hospitals.23 Its use in endodontics has been recently investigated and revealed its ability to remove the smear layer.24 Because of its excellent antimicrobial action in the presence of organic matter, this substance has also been recommended in other applications, such as disinfection of water lines in dental units25 and of acrylic resins.26 These properties justify its evaluation for use as an endodontic irrigant.

A great variety of substances have been evaluated for their action against E. faecalis.7,27,28 Moreover, some studies have suggested the combination of NaOCl with acidic solutions to increase its antibacterial activity.12,13 The present ex vivo study aimed to evaluate the effectiveness of different endodontic irrigating solutions and their combinations against E. faecalis.
MATERIAL AND METHODS

Standardization of the specimens

This study was approved by the Research Ethics Committee of the School of Dentistry of Araraquara—UNESP. One hundred ten extracted single-rooted human teeth with a single straight root canal were selected. Crowns were sectioned with an IsoMet 1000 precision saw (Buehler, Ltda, Lake Bluff, IL) to standardize the length of each specimen at 15 mm.

A #3 Gates-Glidden drill (Dentsply-Maillefer, Bal-laigues, Switzerland) was used to prepare the root canal orifices (3 mm). The root canals were instrumented 1 mm short of the total length up to a #35 Kirschner file (K-file) (Dentsply-Maillefer). Saline solution was used for irrigation. Next, the root canals were filled with 17% trisodium EDTA (Biodinâmica, Ibiporã, PR, Brazil) for 3 minutes, and then washed with 5 mL of saline solution.

The root apices were then sealed with composite resin and all external root surfaces were made impermeable with epoxy adhesive, except for the cervical access. The specimens (n = 15) were randomly divided into seven 24-well cell culture microplates (Corning Incorporated, Corning, NY) and 1 control microplate (n = 5). Specimens were attached to the wells with acrylic resin and sterilized by gamma rays from a cobalt 60 source (EMBRARAD, Cotia, SP, Brazil).

Contamination with \textit{E. faecalis}

The procedures were carried out in a laminar flow chamber (VecoFlow Ltda, Campinas, SP, Brazil). Standardized suspensions of \textit{E. faecalis} ATCC 29,212 were spectrophotometrically obtained ($1.5 \times 10^8$ colony-forming units [CFU]/mL). Tryptic soy broth (TSB; Difco, Detroit, MI) was added to the bacterial suspension at a 1:1 ratio and the root canals were contaminated with 20 µL of this mixture. A sterile cotton pellet was soaked with TSB and placed at the root canal entrances.

The microplates containing the specimens were kept in a microaerophilic environment at 37°C for 21 days. Throughout this period, the root canals received TSB on alternate days by means of a 0.5-mL insulin syringe (Becton Dickinson, Curitiba, PR, Brazil), as described by Cardoso et al.\textsuperscript{28}

Afterward, samples were harvested from each root canal so as to confirm contamination (initial sample) using 3 sterile paper points (Tanari Industrial Ltda, São Paulo, SP, Brazil) and transferred to tubes containing sterile saline. Decimal serial dilutions were made and aliquots were seeded in triplicate onto Petri dishes containing tryptic soy agar (TSA; Difco) and incubated in microaerophilic conditions at 37°C for 48 hours. Bacterial growth was measured by the CFU/mL counts of \textit{E. faecalis}.

Division of the experimental groups

Seven experimental groups were established according to the irrigant used (Table I). In group II (GII) and GIII, the solutions were mixed in a syringe before irrigation. The ratio of each solution was determined by pH analysis: ratios resulting in pH 5 were selected. At this pH level, the antimicrobial activity of NaOCl solutions is enhanced\textsuperscript{29} because of the availability of free chlorine as HOCl.\textsuperscript{14} The specimens in the control group were not submitted to biomechanical preparation.

All root canals in the experimental groups were instrumented up to a #50 K-file. Next, they were instrumented up to a #70 K-file following a step-back technique. At each instrument change, root canals were irrigated with 2 mL of the corresponding irrigant. Total preparation time was standardized at 10 minutes per specimen. Finally, 2 mL of a specific neutralizing agent was used (Table I).

Samples and microbiological analysis

Samples were taken immediately after the root canal instrumentation (postpreparation sample). Decimal dilutions of the suspensions were plated and incubated, similar to the procedures followed after the initial sample. The root canals were then filled with sterile saline and a sterile cotton pellet was placed at the canal entrance. The microplates containing the specimens were covered and incubated once again in a microaero-
phillic environment at 37°C for 7 days, after which a new sample was taken (final sample). Harvesting of samples from the control group (canals that were not prepared) was performed at the same times.

**Statistical analysis**

Results were submitted to logarithmic transformation and analyzed using the GraphPad Prism 3.0 software (San Diego, CA). Analysis of variance (ANOVA) and Tukey tests were used for comparison between the experimental groups. For comparison between the samples within each group, repeated measures ANOVA and the Bonferroni tests were used. The significance level was set at $P < 0.05$.

**RESULTS**

Contamination methodology was confirmed by the recovery of pure *E. faecalis* strains from all teeth in the initial sample performed 21 days after incubation, which revealed similar CFU/mL counts for all groups (Table II).

Comparison among GI, GIII, GV, and GVI at the postpreparation period revealed that these groups had similar results, but had significantly lower counts than the other groups ($P < 0.05$). At the final period, GI, GV, and GVI presented similar results ($P < 0.05$), but differed significantly from the other groups, as shown in Table II.

According to Table III, immediately after biomechanical preparation, all irrigants reduced the number of bacteria inside the root canal. However, at the final sample, a significant increase in the CFU/mL counts was observed in all groups.

The 5 specimens in the control group, which were not instrumented after the contamination period, confirmed the viability of *E. faecalis* throughout the experimental phases.

**DISCUSSION**

The experimental model we adopted allows simulation of endodontic infection in the root canal system and dentin tubules, closely resembling in vivo clinical situations. *E. faecalis* was used because of its high prevalence in cases of endodontic treatment failure and also because of its ability to form biofilm and survive as a monoculture. A 21-day period of contamination allows diffusion of the *E. faecalis* suspension through the dentin tubules, as reported by Berber et al. and Cardoso et al. Seven days after instrumentation, a second sample was taken to verify the permanence of viable bacteria inside the root canal system.

The results of the present study demonstrated that biomechanical preparation using all irrigating solutions significantly reduced the presence of *E. faecalis*. However, an increase in the number of CFU/mL 7 days after instrumentation was observed in all groups. This may be because of biofilm formation, rendering the bacterial cells more resistant than when in planktonic form, besides the persistence of bacteria inside the dentin tubules and ramifications of the main canal. These results are in agreement with those reported by Byström and Sundqvist, who demonstrated in vivo that bacte-

**Table II.** Comparison between the groups in the initial, postpreparation, and final samples (mean and SD of CFU/mL log)

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial</th>
<th>Postpreparation</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI, 2.5% NaOCl</td>
<td>11.00 ± 0.59a</td>
<td>0.00 ± 0.00a</td>
<td>6.40 ± 2.75a</td>
</tr>
<tr>
<td>GII, 2.5% NaOCl + 10% citric</td>
<td>10.85 ± 0.64a</td>
<td>1.47 ± 1.45b</td>
<td>8.89 ± 1.69b</td>
</tr>
<tr>
<td>GIII, 2.5% NaOCl + apple cider vinegar</td>
<td>11.15 ± 0.80a</td>
<td>0.00 ± 0.00a</td>
<td>9.71 ± 1.21b</td>
</tr>
<tr>
<td>GIV, apple cider vinegar</td>
<td>11.13 ± 0.73a</td>
<td>2.06 ± 1.78b</td>
<td>10.47 ± 1.28b</td>
</tr>
<tr>
<td>GV, 2% chlorhexidine</td>
<td>10.65 ± 0.89a</td>
<td>0.00 ± 0.00a</td>
<td>7.77 ± 1.23b</td>
</tr>
<tr>
<td>GVI, 1% peracetic acid</td>
<td>11.07 ± 0.56a</td>
<td>0.00 ± 0.00a</td>
<td>8.01 ± 1.26b</td>
</tr>
<tr>
<td>GVII, saline</td>
<td>11.03 ± 0.70a</td>
<td>2.06 ± 1.78b</td>
<td>9.68 ± 1.30b</td>
</tr>
</tbody>
</table>

Different superscript letters a,b,c indicate statistically significant difference according to the Tukey test ($P < 0.05$).

**Table III.** Comparison between the initial, postpreparation, and final samples within each group (mean CFU/mL log)

<table>
<thead>
<tr>
<th>Sample</th>
<th>GI</th>
<th>GI</th>
<th>GII</th>
<th>GIII</th>
<th>GIV</th>
<th>GV</th>
<th>GVI</th>
<th>GVII</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>11.00a</td>
<td>10.85a</td>
<td>11.15a</td>
<td>11.13a</td>
<td>10.65a</td>
<td>11.07a</td>
<td>11.03a</td>
<td>11.51a</td>
<td></td>
</tr>
<tr>
<td>Postpreparation</td>
<td>0.00b</td>
<td>1.47b</td>
<td>0.00b</td>
<td>2.06b</td>
<td>0.00b</td>
<td>0.00b</td>
<td>0.00b</td>
<td>2.06b</td>
<td>11.02a</td>
</tr>
<tr>
<td>Final</td>
<td>6.40c</td>
<td>8.89c</td>
<td>9.7c</td>
<td>10.47c</td>
<td>7.77c</td>
<td>8.01c</td>
<td>9.68c</td>
<td>10.23a</td>
<td></td>
</tr>
</tbody>
</table>

Different letters indicate statistically significant difference according to the Bonferroni test ($P < 0.05$).
ria remaining after biomechanical preparation can re-colonize the root canals.

NaOCl completely eradicated *E. faecalis* immediately after instrumentation but allowed bacterial growth 7 days later, as previously observed by other authors. The NaOCl + citric acid combination presented lower antibacterial activity than pure NaOCl in both periods. A possible explanation for this would be the release of chlorine gas when the 2 substances were mixed, before irrigating the root canal, as suggested by Baumgartner and Ibay. The NaOCl + apple cider vinegar combination, after 7 days, presented higher CFU/mL counts than NaOCl alone. Thus, association of NaOCl with acidic substances did not prove advantageous, contrasting with the report by Camps et al. It should be pointed out, however, that those authors used a higher concentration of NaOCl (10%) in association with chloridric acid, to obtain a 2.5% NaOCl solution with neutral pH.

Plain apple cider vinegar promoted a significant reduction in the numbers of *E. faecalis* following instrumentation, but the final bacterial counts were similar to the initial values.

The efficacy of 2% chlorhexidine against *E. faecalis* has been previously demonstrated, both in vitro and in vivo. Our results demonstrate similar antibacterial activity for 2% chlorhexidine and 2.5% NaOCl, contrasting with the findings by Dametto et al., who reported absence of bacteria 7 days after preparation and irrigation with chlorhexidine. However, these authors allowed an incubation period of only 7 days after inoculation, which may have prevented greater penetration of *E. faecalis* in the root canal system.

Peracetic acid solutions in concentrations ranging from 0.2% to 10.0% have been tested. The efficacy of 2% chlorhexidine used as a root canal irrigating solution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:e66-73.


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