Evaluation of antimicrobial effectiveness of two endodontic irrigation solutions on the microbial reduction of Enterococcus faecalis in infected root canals (in vitro study)

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Abstract

**Objective:** The aim of this study was to evaluate the ability of a new irrigant solution QMix, in the microbial reduction of Enterococcus faecalis from infected root canals in comparison with sodium hypochlorite 5.25%.

**Materials and Methods:** 40 extracted mandibular premolars with single root canal have been prepared with rotary protaper instrument until F3 and irrigated with saline, and then they were sterilized by moist heat sterilization at temperatures 121°C for 20 min. After that, they were injected with 50 μ of Enterococcus faecalis suspension in the density of 10^8, and then the root canals were incubated in a temperature of 37°C for 7 days in aerobic and anaerobic conditions. Next, the root canals were divided into two groups. Group 1 is irrigated with sodium hypochlorite 5.25%, Group 2 is irrigated with QMix. Each specimen was irrigated for 40 s, and the solution was left inside the canal for 30 s. The bacterial swabs were taken by sterilized paper points in two stages; the first one was after the end of incubation period, and the second one was immediately after the irrigation. Student’s t-test and Chi-square test were used to compare bacterial reduction Log(10) values between the groups.

**Results:** Sodium hypochlorite was more effective than QMix (P < 0.05). There were no significant differences in the bacterial reduction Log(10) percent between anaerobically and aerobically incubated roots in sodium hypochlorite 5.25% solution group (P > 0.05), whereas bacterial reduction Log(10) percent in anaerobically incubated roots were lower than those of aerobically incubated roots in Qmix solution group (P < 0.05). Furthermore, the percentage of full cleansing in the sodium hypochlorite group was greater than those of Qmix solution group (P < 0.05). However, the percentage of full cleansing in the QMix group was zero.

**Conclusions:** Sodium hypochlorite 5.25%, had clear ability on the microbial reduction of E. faecalis in comparison with the new irrigant QMix.

Introduction

Bacteria and their products play a key role in the initiation and continuation of the pulp and periapical diseases. Thus, eradicating them and preventing their resolution are the goal in any successful endodontic treatment in the short- and long-term. Opportunistic bacteria invade the root canal system due to loss of the body ability to defend in areas of necrotic tissue and the availability of low-oxygen environment. This provides a favorable environment for the occurrence of infection in the root canal.

Some studies have shown that the mechanical preparation of the canal which based on the use of k-files only or rotary instruments is not sufficient in eliminating these bacteria, due to the anatomical complexities of root canal which make these bacteria be far away from the files access. Thus, endodontic treatment failure as a whole in the long-term.

Studies have shown the need to support the mechanical preparation by chemical materials which are capable of penetrating most of the anatomical complexities of the root canals and eradicate these bacteria, the irrigation solution plays an important role in debridement and disinfect the root.

Keywords
Bacteria, Enterococcus faecalis, Irrigation solution, QMix, root canal irrigation, sodium hypochlorite

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Received 14 November 2015
Accepted 25 December 2015
doi: 10.15713/ins.idmjar.33
canal system and is considered an important stage of the cleaning and shaping procedures of the root canal. Although most studies have proven the effectiveness of sodium hypochlorite in the completion of this task, some showed insufficiency of this solution in eliminating the bacterium (Enterococcus faecalis) which is one of the most common types of microorganism isolated from teeth which have post-treatment disease. This is because of its high potential of camouflage and resistance, where it is the most resistant to harsh environmental conditions, and to many medicaments. It is also able to invade the dentinal tubules. This resistant is attributed to the ability to form a complex structure called biofilm. In addition to the difficulty of removing this biofilm and other bacterial structures that colonizing root canal, the presence of dentin residues and the debris from root canal preparation provide hiding places to previous structures like smear layer which make this more difficult to remove.

As a result of that the focus on raising the effectiveness of the irrigation solution used in chemomechanical preparation of the canal has increased, the attempts began to get the ideal irrigation solution.

Surface-active agents have been added to several different types of irrigants to lower their surface tension and to improve their penetration into the root canal. Furthermore, It had been noted that the use of a single solution is not enough to achieve the objectives of canal irrigation, but the use of two solutions at least like the alternating use of chelating agents and antimicrobial solutions through the preparation helps in achieving better disinfection, such as QMiX (Dentsply Tulsa Dental) which is one of the new combination products introduced for root canal irrigation. QMiX contains ethylenediaminetetraacetic acid (EDTA), chlorhexidine, and a detergent and comes as a ready-to-use clear solution.

Materials and Methods

The antimicrobial effectiveness of the following irrigants was studied:

1. Sodium hypochlorite 5.25% (NaOCl)
2. Qmix.

Specimen preparation

A total of 40 human mandibular premolars with a fully formed apex and single root canal were checked by radiographs, without internal resorption, calcifications or root dilacerations and with no other anatomical or pathological alterations were selected.

The crowns were sectioned to standardize the root length to 17 mm. Root canals were enlarged using k flexo file (Dentsply Maillefer) size 15, 20 until the instrument tip was visualized at the apical foramen.

Each specimen was prepared using protaper instrument until F3 under irrigation with saline. After that, each specimen was irrigated with 40 ml sodium hypochlorite 5.25% and alternating with 10 ml EDTA 17% using NaviTip 31 gauge with Double Sideport Irrigator Tip to remove smear layer so that the total time of irrigation 10 min per specimen.

Then, the roots were dried, composite resin was used to seal the apex, and nail polish was applied around the root surface. Cotton pellets were placed onto the canal orifice and then sealed with temporary filling. To make both handling and identification easier, the teeth were mounted vertically in acrylic resin, after setting of the acrylic resin, the temporary filling and pellet were removed. The tubes were placed in autoclave sachets and then autoclaved for 15 min at 121°C, five teeth were chosen randomly, bacterial swabs were taken of them so as to ensure the sterility of the specimens where the cultures were negative.

Infection of the specimens

E. faecalis was isolated clinically; microbial strains were confirmed by Gram’s stain and by colonial and growth characteristics. The bacterial suspension prepared in density of 10^8 colony-forming units/ml in sterile saline solution by using McFarland standard tubes No. 1.

Each root canal was filled with 50 μ of E. faecalis suspension via their orifice using sterile micropipettes. Then, the roots were divided into two equal groups (n = 20) where the first group was incubated aerobically and the second group was incubated anaerobically at 37°C, for 7 days.

Experimental groups

After the contamination, each of the aerobic and anaerobic group was divided randomly into two groups (n = 10) according to the intracanal irrigant used, NaOCl 5, 25% and Qmix solution.

The initial count of bacterial unites

After the end of incubation period, the initial bacterial swab was taken from each root canal as follows:

The root canal was filled with sterile saline solution using a sterile disposable injector. Then, a sterile size 25 H file was inserted into the root canal, and the canal walls were slightly touched with an in-out motion circumferentially to remove the unattached cells. Moreover, Swab was taken by 3-paper points compatible with the size of the preparation (Protaper F3) placed to the working length. Each paper point remained in the canal for 1 min. Paper points were transferred to eppendorf tube containing 2 ml of sterile saline and vortexed for 1 min. Paper points were plated onto Mueller Hinton agar plates for aerobic culture and onto Thioglycollate agar plates for anaerobic culture, and then the plates were incubated at 37°C for 24 h. Colony-forming units grown were counted, and a log transformation was calculated.

Irrigation protocol

The root canals were irrigated for 40 s by 2 ml of irrigation solution (sodium hypochlorite 5.25% and Qmix according to the study groups) at room temperature using NaviTip 31 gauge with Double Sideport Irrigator Tip, which inserted into the root canal 2 mm before the apex.
Then, the irrigation solution was left in the root canal for 30 s before removal with 10 ml of saline solution to remove its effects from the root canal.

**The count of bacterial unites after the irrigation**

After the completion of the irrigation protocol, root canals were dried onto working length, and then were filled with 5 ml saline solution, and sterile H-file size 25 was inserted into the root canal and the canal walls were slightly touched with an in-out motion circumferentially for 15 s and the same culturing procedures were applied as for the initial sampling.

Data were analyzed using Student’s t-test, and Chi-square test.

**Results**

**Sample description**

The sample consisted of 40 roots divided into two main distinct equal groups according to the incubation method (anaerobically incubated roots group, aerobically incubated roots group); each one of the main groups was divided into two subgroups according to studied irrigation solution (sodium hypochlorite 5.25% solution group, Qmix solution group) [Graph 1].

- Before the irrigation there were no significant differences in the mean value of bacterial reduction Log(10) between sodium hypochlorite 5.25% solution group and Qmix solution group \((P > 0.05)\), but after the irrigation the bacterial reduction Log(10) values in the both groups were lower than those before the irrigation [Table 1 and Graph 2].
- Bacterial reduction Log(10) percent (absolute values) in the sodium hypochlorite 5.25% solution group were greater than those of Qmix solution group whatever the studied incubation method shown in Table 2 and Graph 3 \((P < 0.05)\).
- There were no significant differences in the mean value of bacterial reduction Log(10) percent between anaerobically incubated roots group and aerobically incubated roots group in sodium hypochlorite 5.25% solution group \((P > 0.05)\), whereas bacterial reduction Log(10) percent in anaerobically incubated roots group were lower than those of aerobically incubated roots group in Qmix solution group \((P < 0.05)\) [Table 3 and Graph 4].

**Table 1:** Independent samples student’s t-test results to know if there were significant differences in bacterial no Log(10) values between sodium hypochlorite 5.25% solution group and Qmix solution group according to studied stage and incubation method

<table>
<thead>
<tr>
<th>The incubation method</th>
<th>Studied stage</th>
<th>t value</th>
<th>df</th>
<th>Mean difference</th>
<th>Standard error difference</th>
<th>P value</th>
<th>Significant difference?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobically incubated roots</td>
<td>Before irrigation</td>
<td>−1.467</td>
<td>18</td>
<td>−0.39</td>
<td>0.26</td>
<td>0.160</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>After irrigation</td>
<td>−2.832</td>
<td>18</td>
<td>−1.55</td>
<td>0.55</td>
<td>0.011</td>
<td>Yes</td>
</tr>
<tr>
<td>Aerobically incubated roots</td>
<td>Before irrigation</td>
<td>0.141</td>
<td>18</td>
<td>0.01</td>
<td>0.09</td>
<td>0.890</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>After irrigation</td>
<td>−7.075</td>
<td>18</td>
<td>−2.11</td>
<td>0.30</td>
<td>0.000</td>
<td>Yes</td>
</tr>
<tr>
<td>All roots</td>
<td>Before irrigation</td>
<td>−1.047</td>
<td>38</td>
<td>−0.19</td>
<td>0.18</td>
<td>0.302</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>After irrigation</td>
<td>−5.670</td>
<td>38</td>
<td>−1.83</td>
<td>0.32</td>
<td>0.000</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Effectiveness of two irrigation solution on Enterococcus faecalis

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Full cleansing occurrence percents in sodium hypochlorite 5.25% solution group were greater than those of Qmix solution group whatever the studied incubation method shown in Table 4 and Graph 5 ($P < 0.05$).

### Discussion

*E. faecalis* is a non-fastidious, easy-to-grow facultative anaerobic Gram-positive Coccus[23] that penetrates into dentinal tubules, leading to gross infection and attaches itself to the collagen in the presence of human serum, keeping its survival methods intact.[24]

This microorganism was chosen as the bacteriological marker in this study because of its reported resistance to chemomechanical procedures. It is often involved in persistent endodontic infections and periapical inflammation and is often found existing in monocultures.[8] It is one of the most resistant species found in the oral cavity, having the ability to survive even under unusual environmental stresses, such as low nutrient availability and because it is relatively easy to culture and manipulate.[25] It also has been used in previous studies on the efficacy of irrigant solutions and intracanal medications[26] especially for its high level of resistance to a wide range of antimicrobial agents.[27]

Since anatomy of the root canals may affect the results of microbiologic load, similar mandibular premolar teeth were used in order to standardize the specimens.

Incubation periods vary between 1 day and 1 week in different studies.[25,28] The root canals infected with *E. faecalis* were incubated for 1 week in this study.

Bacteriological sampling was accomplished using sterile paper points. Sampling with paper points has limitations because only the microorganisms that are in the root canal can be sampled while those located inside the dentinal tubules cannot be detected.[29] In this study, before sampling the hand instrument was used with pumping motion along the root canal to allow for a more predictable sampling of narrow canal recesses.

The results of the present study revealed that the initial bacterial load was homogeneous in the experimental groups whatever the studied incubation method.

### Table 2: Independent samples student's $t$-test results to know if there were significant differences in bacterial no reduction Log(10) percent values between sodium hypochlorite 5.25% solution group and Qmix solution group according to incubation method

<table>
<thead>
<tr>
<th>The incubation method</th>
<th>$t$ value</th>
<th>df</th>
<th>Mean difference</th>
<th>Standard error difference</th>
<th>$P$ value</th>
<th>Significant difference?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobically incubated roots</td>
<td>−2.992</td>
<td>18</td>
<td>−34.97</td>
<td>11.69</td>
<td>0.008</td>
<td>Yes</td>
</tr>
<tr>
<td>Aerobically incubated roots</td>
<td>−7.056</td>
<td>18</td>
<td>−58.55</td>
<td>8.30</td>
<td>0.000</td>
<td>Yes</td>
</tr>
<tr>
<td>All roots</td>
<td>−6.442</td>
<td>38</td>
<td>−46.76</td>
<td>7.26</td>
<td>0.000</td>
<td>Yes</td>
</tr>
</tbody>
</table>

### Table 3: Student's $t$-test results in bacterial no reduction Log(10) percent values between anaerobically incubated roots group and aerobically incubated roots group according to studied irrigation solution

<table>
<thead>
<tr>
<th>Studied irrigation solution</th>
<th>$t$ value</th>
<th>df</th>
<th>Mean difference</th>
<th>Standard error difference</th>
<th>$P$ value</th>
<th>Significant difference?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hypochlorite 5.25% solution</td>
<td>1.092</td>
<td>18</td>
<td>15.53</td>
<td>14.22</td>
<td>0.289</td>
<td>No</td>
</tr>
<tr>
<td>Qmix solution group</td>
<td>−4.415</td>
<td>18</td>
<td>−8.05</td>
<td>1.82</td>
<td>0.000</td>
<td>Yes</td>
</tr>
</tbody>
</table>

### Table 4: Chi-square test results in full cleansing occurrence between sodium hypochlorite 5.25% solution group and Qmix solution group according to the incubation method

<table>
<thead>
<tr>
<th>Incubation method</th>
<th>Chi-square value</th>
<th>df</th>
<th>$P$ value</th>
<th>Significant difference?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobically incubated roots</td>
<td>6.667</td>
<td>1</td>
<td>0.0098</td>
<td>Yes</td>
</tr>
<tr>
<td>Aerobically incubated roots</td>
<td>8.571</td>
<td>1</td>
<td>0.003</td>
<td>Yes</td>
</tr>
<tr>
<td>All roots</td>
<td>15.172</td>
<td>1</td>
<td>0.000</td>
<td>Yes</td>
</tr>
</tbody>
</table>

### Graph 4: Average of bacterial no reduction Log(10) percent according to the incubation method and studied irrigation solution

### Graph 5: Percent of full cleansing occurrence according to studied irrigation solution and incubation method
Furthermore, the results demonstrated that sodium hypochlorite 5.25% solution was more effective than QMix solution \((P < 0.05)\). And there were no significant differences in the bacterial reduction \(\log(10)\) percent between anaerobically and aerobically incubated roots in sodium hypochlorite 5.25% solution group \((P > 0.05)\), whereas bacterial reduction \(\log(10)\) in anaerobically incubated roots were lower than those of aerobically incubated roots in Qmix solution group \((P < 0.05)\). Moreover, the percentage of full cleansing in the sodium hypochlorite group was greater than those of Qmix solution group \((P < 0.05)\). However, the percentage of full cleansing in the QMix group was zero.

This superiority of NaOCl solution on the microbial reduction is attributed to its characteristics where NaOCl is a very reactive oxidant that presents a well-documented dissolution and disorganization effect against biofilms;\(^{[30]}\) while the poor results of QMix solution may possibly be explained by that the combination between chlorhexidine and EDTA leads to reduce the antibacterial effect of this new solution, also the total time of irrigation in this study was 1 min and 10 s so maybe it needs more time to achieve its effect.

In this study, The root canals infected with \(E. faecalis\) were incubated for 1 week, the total time of irrigation was 1 min and 10 s and the total amount of irrigation solution was 2 ml, so the duration of incubation, the irrigation time, and the amount of irrigation solution are very important factors to notice when we compare between studies, our results harmonize with the results of other studies which found that NaOCL was more effective than QMix against \(E. faecalis\) at the 1\(^{st}\) min of irrigation.\(^{[16,19]}\) Also that NaOCL was more effective than Qmix against \(E. faecalis\) biofilms.\(^{[31]}\)

**Conclusion**

Under the conditions of this study, the results suggest that irrigation with sodium hypochlorite 5.25% was more effective in reducing the bacterial loads of \(E. faecalis\) than Qmix.

**References**


