An Invitro Study of Micro10 Solution's Capability of Endodontic Hand Instruments Sterilization

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ABSTRACT

Introduction: Microorganisms have an essential role in pulpo-periapical pathosis, therefore obtaining a successful treatment has a direct relationship with their elimination. According to previous studies, the most reliable method of sterilization is autoclave, but in some instances there are limitations for using this method, and we need an alternative. One of the most popular and widespread materials in this field is Micro10 solution. The aim of this research is to determine the sterilization effect of Micro10 solution on endodontic hand instruments.

Methods: 1200 endodontic hand files were selected for this study, (15 groups of 80 files). Each bacterial group was prepared in 0.5 Mc Farland standard concentration (1.5 ×10⁸). Every 5 groups of files were contaminated with one of bacterial samples. Then each group of contaminated files underwent sterilization process, using one of the following methods: a) Application of autoclave, b) Application of 2% Micro10 solution, in 15 minutes, c) Application of 2% Micro10 solution, in 60 minutes, d) Application of 10% Micro10 solution, in 15 minutes, and e) Application of 10% Micro10 solution, in 60 minutes.

Results: The results of this study indicated that only in group 1 (autoclave) all of samples were sterile, but in other groups sterility diminished to 82.1% (group 2), 83.3% (group 3), 90.8% (group 4), and 93.3% (group 5).

Conclusion: Only autoclave is a reliable method for obtaining a sterile condition and Micro10 solution at the most is just a good disinfectant, especially in 10% concentration. Also we concluded that the concentration of Micro10 solution has a direct effect on its killing ability of bacteria, while duration of process (60 minutes versus 15 minutes) has no effect.

Key words: Sterilization, Micro10 Solution, Disinfectant

Introduction

Because of basic role of microorganisms in initiating pulpo-periapical lesions, a successful dental treatment depends on efforts to eliminate them. Therefore infection control has a significant importance in endodontics. Different methods of sterilization have been introduced and applied in endodontics. According to previous studies, the most reliable method of sterilization is autoclave, but in some instances there are some limitations for using this method, and we need an alternative. Till today, a variety of methods have been employed for sterilization of endodontic hand instruments; one of these methods is application of chemical solutions which contain quaternary ammonium compounds are of them, which are effective on gram positive bacteria and more or less, on other pathogens, but ADA omitted this material from the approved disinfectants. The alcohol added to new products has given a wide spread antibacterial effect to these

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materials. According to Unident (Swiss), the Micro10 commercial solution is effective on bacteria, fungi, tuberculosis bacillus, and viruses. Micro10 is an inexpensive material, with easy and rapid usage. Widespread using of Micro10, necessitate to perform researches on sterilizing effect of this agent. The general purpose of this research is to determine the sterilizing effect of Micro10 solution on endodontic hand instruments by use of four bacterial species and one spore.

Materials & Methods
Micro10, a material with the base of quaternary ammonium, is manufactured by Unident (Swiss). From the stand point of physical properties, it is an odorless material with yellowish color which according to its manufacturer, is bactericidal, fungicidal, tuberculcidal, and virucidal. That is effective on HIV and HBV. In this study, we used it in 2% and 10% concentrations. These bacterial strains were selected for this study:
- a. Streptococcus viridans
- b. Staphylococcus aureos
- c. Neisseria
- d. Psudomonas aeroginosa
- e. Bacillus saprophyte spore

The first three bacteria formed the first group in the form of a bacterial suspension, something like oral bacterial flora. Psudomonas was the bacterium in group 2, and Bacillus saprophyte was the bacteria of group 3.

Bacteria of each group were was prepared in 0.5 McFarland (1.5 × 10^8) standard concentration. Culture medias in this study were:
- 1. EMB-agar
- 2. Blood agar
- 3. BHI 4.TSI

1200 endodontic files were divided in 15 groups and each group contained 80 files. All files underwent sterilization cycle, in 15 psi, 15 minutes, and 121 centigrade degree by autoclave. Then every 5 groups of files were contaminated with one bacterial group, as bellow:
- groups 1 to 5 contaminated with oral bacterial suspension,
- groups 6 to 10 contaminated with Psudomonas aeroginosa,
- groups 11 to 15 contaminated with Bacillus saprophyte spore (Table 1).

Then each group was sterilized using 1 of 5 following:
- 1-use of autoclave,
- 2-use of Micro10 solution (2%) in 15 minutes,
- 3-use of Micro10 solution (2%) in 60 minutes,
- 4-use of Micro10 solution (10%) in 15 minutes,
- 5-use of Micro10 solution (10%) in 60 minutes.

The files of groups 1, 6 and 11 sterilized by autoclave, files of groups 2, 7, and 12 sterilized with method 2, files of groups 3, 8, and 13 sterilized with method 3, files of groups 4, 9, and 14 sterilized with method 4, and files of groups 5, 10, and 15 sterilized with method 5.

<table>
<thead>
<tr>
<th>Microbial Groups</th>
<th>Sterilization Methods</th>
<th>Autoclave</th>
<th>Micro10 2% - 15min</th>
<th>Micro10 2% - 60min</th>
<th>Micro10 10% - 15min</th>
<th>Micro10 10% - 60min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral flora</td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Psudomonas</td>
<td></td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>aeroginosa</td>
<td></td>
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<td>pore</td>
<td></td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 1: Grouping of files in this study

Then, under sterile conditions, each file was immersed in BHI fluid culture media. Each culture gave a code and then was incubated for 48 hours in 37 centigrade degree, and then the results were recorded. Turbidity of culture media indicated that the file is not sterile, and lack of turbidity indicated that the file is sterile and the method is successful. After the results of BHI were recorded, a specimen taken from the fluid of each sample was brought to Blood agar solid culture media, and each specimen received the previous code. Each culture was incubated for 48 hours in 37 centigrade degree, and then the results were recorded. Lack of colony formation indicated that the specimen was sterile, and formation of colony indicated that the specimen was not sterile and the method was failed. From the BHI fluid culture media of groups 6 to 10 (Pseudomonas aeroginosa), a specimen was taken from each sample and brought to EMB agar culture media, and the results were recorded. From the positive results of EMB agar of groups 6 to 10, specimens were taken by loop, and were brought to TSI culture media, and the results were recorded after 24 hours.

The presence of dark red color indicated that the Pseudomonas aeroginosa was present, and the presence of acidic color in entire media indicated that this bacteria was absent. After computerizing all data, using chi-square and fisher tests, the results were analyzed.

Results
These results are obtained after data statistical analysis.
1. Sterility verification of 240 files by autoclave showed that 100% of samples were sterile.
2. Sterility verification of 240 files with 2% Micro10 solution in 15 minutes showed that 82.1% of samples were sterile.
3. Sterility verification of 240 files with 2% Micro10 solution in 60 minutes showed that 83.3% of samples were sterile.
4. Sterility verification of 240 files with 10% Micro10 solution in 15 minutes showed that 90.8% of samples were sterile.
5. Sterility verification of 240 files with 10% Micro10 solution in 60 minutes showed that 93.3% of samples were sterile (Diagram 1).

Diagram 1:
Discussion

Previous researches have indicated the role of bacteria in pathogenesis of pulpoperaipical diseases. Studies performed by Miller $^5$, Melville and Brich $^6$, Boling and Robinson $^7$, Kakehashi et al $^8$, and Krozen $^9$ indicated the important role of microorganisms in pathogenesis of these diseases. Winkler and Van $^7$ showed in their study that the main bacterium found in infectious root canals was Streptococcus and Micrococcies. Studies of Kantz and Henry $^8$, and Haapsalo $^9$ indicated that anaerobic bacteria exist in 90% of contaminated root canals. Mollar's $^{10}$ study showed the importance of bacteria in pulpoperaipical pathosis. Fabiricus $^{11}$ noted that pulpal infection and periapical pathosis are the sequela of multibacterial factors. So, for achieving a successful treatment, all efforts must be made to eliminate all microorganisms completely, in fact, achieving “Sterilization”. Sterilization means elimination of all microorganisms and also spores. Studies show that only the “under pressure Steam” (autoclave) is the effective method for elimination of all microorganisms. The results of this study indicated that only in autoclave group, all of samples were sterile, and in other groups sterility diminished significantly. Since in sterilization we want 100 percent elimination of microorganisms and spores, any method which is unable to achieve this is not a method of choice.

According to the results of present study, Micro10 solution is unable to achieve 100 percent sterility, so we can not place it in the category of sterilizing agents, but it seems that Micro10 is a very good disinfectant, especially in 10 percent concentration when applied for 60 minutes. Also, the results of this study indicates that the duration of Micro10 application has no effect on its level of sterility.

Diagram 2 shows that the least sterilization capability of Micro10 solution is in group 2 of bacteria (pseudomonas aeruginosa). The reason is high resistance of this microorganism due to lack of permeability of external membrane of bacterium which acts like a barrier against large molecules. The least sterilization capability of Micro10 solution is in group 2 of sterilization methods (use of Micro10 solution 2% in 15 minutes).

All results of present study indicates that all of samples were sterile only in autoclave method and other methods failed to achieve sterility, so chemical sterilization is not a good substitute for autoclave. Micro10 solution could be used for disinfecting the working surfaces, tables, and also for instruments and appliances not resistant to heat or steam. An Invivo study is recommended to prove these results clinically.

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