Bacterial Leakage in Roots Filled With Different Medicaments and Sealed With Cavit

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Abstract
The aim of this study was to evaluate the time required by four different root canal medications coupled with the temporary filling material Cavit (ESPE, Seefeld, Germany) to prevent penetration of bacteria into the root canal. There were 145 roots prepared in a standardized manner. Four groups with 15 samples each were treated with calcium hydroxide (Ca(OH)₂), a 5% chlorhexidine gel (CHX), a chloromono-campherphenolic compound (ChKM), and Ledermix (LM), respectively, and sealed with Cavit. Four control groups contained identical medications but the roots were left unsealed. The 25 remaining roots served as additional controls. A standard setup for bacterial leakage studies was chosen with Staphylococcus epidermidis as test strain. Cavit application resulted in a significantly better seal compared with the unsealed groups. In the Cavit-sealed groups, all groups differed significantly from one another except for the CHX and the ChKM groups. The Ca(OH)₂ medicated roots provided the longest protection (median of 36 days), followed by the Ledermix-group (27 days) and the CHX (18 days) or ChKM groups (19 days). It may be concluded that Cavit-sealed and medicated root canals do not provide adequate protection against bacterial leakage for more than 1 month.

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Key Words
Bacterial penetration, calcium hydroxide, cavit, chlorhexidine, ledermix, phenolic derivative, temporary medication

Materials and Methods
There were 145 human maxillary canines used for this experiment. After separation of the crown, the roots were coronally ground to a uniform length of 16 mm. Standardized cavities, mimicking clinical access cavities, 5 mm in depth and 2.5 mm in diameter were drilled into the coronal parts of the roots. The canals were instrumented to an ISO #60 file at 15 mm length while being irrigated with copious amounts of 2.5% NaOCl. The apical opening was standardized by placing an ISO #30 instrument 1 mm beyond the apex. The smear layer was removed with a 2-minute rinse of 15% EDTA followed by a final flush of NaOCl. The roots were randomly assigned to four test groups with 15 samples each and seven control groups, five of them with 15 samples and two with five samples each.

All roots were sterilized in ethylene-oxide and further treated under sterile conditions. In test groups 1 to 4, the root canals were filled with four different temporary dressings: Group 1 (Ca(OH)₂/Cav) received a fresh mix of Ca(OH)₂, group 2 (LM/Cav) Ledermix paste (Lederle, Wolfratshausen, Germany), group 3 (CHX/Cav) an experimental 5% CHX-methylcellulose gel, and in group 4 (ChKM/Cav), a cotton pellet soaked in a campher monochlorphenolic compound with an additive of menthol (Walkhoff’s...
ChKM: Haupt, Würzburg, Germany) was placed in the access cavity. All test groups were provided with a small cotton pellet at the canal orifices and coronally sealed with Cavit. Control groups 5 to 8 (Ca(OH)_2, LM, CHX and ChKM) were each filled with the same medication as in the experimental groups and a small cotton pellet was inserted into the access cavity. The root canals were, however, left unsealed. There were 15 root canals sealed coronally with Cavit only. Five root canals were left empty and served as positive controls, whereas five other root canals were entirely covered with sticky wax, serving as negative controls.

The roots were placed between two chambers according to a former experimental setup described by Barthel et al. (11). The upper chamber contained a streptomycin resistant Staphylococcus epidermidis strain (1 × 10^8 CFU/ml), as test strain. To minimize the possibility of contamination, the lower chamber was filled with a sterile clear soy broth containing 0.125 mg/ml streptomycinsulfate. Occurrence of turbidity was checked on a daily basis. Turbidity in the lower chamber indicated a leaking root. Upon appearance of the turbidity, the sample was opened and vitality and conformity of bacteria with the upper chamber were checked by incubating liquid of both chambers on Columbia agar. The experiment was conducted until all samples except for the negative controls leaked.

After gathering the data, they were statistically confirmed as being nonparametric with the Kolgomorov-Smirnov test. The average day of leakage was determined for each group. The Kruskal Wallis test was applied to detect the presence of statistically significant differences among the groups. The single groups were tested against one another by employing the Mann-Whitney U test. In addition, every week the cumulative number of leaking samples was recorded per group. The χ² test was calculated to detect significant differences for the number of leaking samples per week and group. The level of significance was set at p < 0.05.

Results

The experiment was terminated after 6 weeks. By then, all samples except for the negative controls had leaked. Positive (empty) controls leaked after 1 day. All turbid bottom chambers were bacteria-positive with S. epidermidis. The number of leaking samples per group and the first and last day of leakage can be seen in Table 1.

All groups differed significantly from one another with regard to the mean day of leakage in the test groups, except for the CHX/Cav and the ChKM/Cav groups. Ca(OH)_2/Cav showed the longest protection followed by LM/Cav and CHX/Cav or ChKM/Cav. The same statistical significances applied to the unsealed control groups, in which, once again, the Ca(OH)_2 samples showed longest protection. In general, Cavit application resulted in a significantly better seal when comparing the unsealed with the sealed groups.

The cumulative numbers of leaking samples per week can be seen in Figs. 1 and 2. In the sealed and medicated groups, Ca(OH)_2/Cav and LM/Cav showed significantly fewer leaking samples in week 3 compared to the CHX/Cav and the ChKM/Cav groups. In weeks 4 and 5 Ca(OH)_2/Cav was significantly superior to the two aforementioned groups. In unsealed groups, Ca(OH)_2 and LM groups showed significantly fewer leaking samples during the first 2 weeks when compared to the other two groups. Besides the above-mentioned no significant differences in the number of leaking samples was observed.

Discussion

Intracanal dressings may be applied to support chemo-mechanical debridement (12, 13). When administered because of lack of time

### Table 1. Days of leakage per group

<table>
<thead>
<tr>
<th>Dressing</th>
<th>Test Groups Leaking Between Day</th>
<th>Median Day of Leakage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca(OH)_2/Cav</td>
<td>27 and 47</td>
<td>36</td>
</tr>
<tr>
<td>LM/Cav</td>
<td>20 and 37</td>
<td>27</td>
</tr>
<tr>
<td>ChKM/Cavit</td>
<td>10 and 21</td>
<td>n.s. 19</td>
</tr>
<tr>
<td>CHX/Cavit</td>
<td>11 and 21</td>
<td>18</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dressing</th>
<th>Control Groups Leaking Between Day</th>
<th>Median Day of Leakage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca(OH)_2</td>
<td>12 and 24</td>
<td>19</td>
</tr>
<tr>
<td>Ledermix</td>
<td>1 and 20</td>
<td>14</td>
</tr>
<tr>
<td>ChKM</td>
<td>All on day 1</td>
<td>n.s. 1</td>
</tr>
<tr>
<td>CHX</td>
<td>All on day 1</td>
<td>1</td>
</tr>
<tr>
<td>Cavit</td>
<td>10 and 20</td>
<td>17</td>
</tr>
<tr>
<td>Positive Controls</td>
<td>All on day 1</td>
<td>1</td>
</tr>
<tr>
<td>Negative Controls</td>
<td>No leakage</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 1. Unsealed (control) samples: number of leaking samples per group, cumulative values per week.

Figure 2. Corronally sealed samples: number of leaking samples per group, cumulative values per week.
during endodontic treatment they serve to occupy root canal space and therefore do not leave room for bacterial multiplication (14). Depending on the individual treatment protocol, medications may be applied for days, weeks, or in case of apexogenesis even months. Hence, besides disinfection, it is necessary for the medications to maintain an environment low in bacterial count. In the present study, Ca(OH)$_2$ has been used as standard medication. According to a recent meta-analysis it has been designated as the best root canal medication available (15). As CHX has often been discussed as a replacement or adjunct medication it was added to the study protocol (16, 17). Because Ledermix is frequently used in Europe and Australia it was also incorporated into the study. Finally, owing to its popularity in private practices, although it has been proven to be inferior to Ca(OH)$_2$ in an in vivo study by Bystroim et al. (12), chloromonom-camphorphenolic compound was also included in the study. The exact formula of the presently used Walkhoff solution (ChKM) was slightly different from the one used by Bystroim et al. in their study. It contained 217 mg/g 4-chlorophenol, 712 mg/g camphor, and 17 mg/g menthol in alcohol. The solution used in the Bystroim study (CMCP) contained 30% monochlorophenol, 60% camphor, and 10% alcohol. Whether this difference in formulation has a significant impact on the outcome of disinfection has not yet been proven.

All intracanal dressings were applied with a lentulo spiral, except for CHX, which has a much lower viscosity. Hence, a cotton pellet was soaked in the volatile solution and placed in the cavity, thereby negating its function of occupying the root canal space to suppress bacterial multiplication. This, in addition to it being highly volatile could give reason for its inferior performance in this study. Studies show that one day after application of CMCP, only up to 10% of it remains in the root canal system (18–20). This result may be similar with the ChKM solution.

CHX is known to have a good antibacterial effect (21–23). However, in the present study the antibacterial protection attributed was similar to the one of ChKM. This may be a result of the fact that dentin reduces the disinfecting effect of CHX to a certain extent (24). Furthermore, a substantivity effect as discussed in earlier studies could not be confirmed by this study (25, 26).

The long-term antimicrobial effect of Ledermix has been questioned (27). In the present study it was found to be superior to CHX and ChKM in preventing bacterial intrusion. Ledermix does not set after application, however, it does create some kind of barrier by drying out. This barrier may be helpful in prolonging the protection. Additionally, only one strain of bacteria was tested in this study. It may be assumed that Ledermix has a very good antibacterial effect against this strain. In former studies, Cavit has been shown to have a weak seal against bacterial penetration (10, 11). In this study, four groups were dressed with the medicaments and left unssealed as controls. The four test groups were coronally sealed. In this manner the sole effect of Cavit could be determined. By placing Cavit into the coronal cavity, average bacterial penetration could be prolonged for 13 to 18 days. This duration could have been increased by avoiding the use of cotton pellets underneath the Cavit filling. Even when placed with utmost care, it is inevitable to prevent extrusion of even tiniest cotton fibers from the cavity providing a pathway for bacteria into the root canal system. Prior knowledge of this drawback encouraged the authors to place the pellets as carefully as possible. This procedure was chosen because is a common practice rendering an easy access to the root canal in the following appointment.

It may be concluded that in terms of providing protection against bacterial penetration Ca(OH)$_2$ presents itself as the best root canal medication. Coronal seal with Cavit prolongs the protection significantly, nevertheless, an adequate seal cannot be provided for more than 1 month. Other coronal sealing materials such as glass-ionomer cement or adhesively applied composite fillings may enhance the seal for longer time intervals. The camphorated monochlorphenolic compound showed the worst results.

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References


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