ANTIMICROBIAL ACTIVITY OF ENDODONTIC SEALERS BASED ON CALCIUM HYDROXIDE AND MTA

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ABSTRACT
The aim of this study was to evaluate the antimicrobial activity of a new root canal sealer containing calcium hydroxide (Acroseal) and the root canal sealer based on MTA (Endo CPM Sealer), in comparison with traditional sealers (Sealapex, Sealer 26 and Intrafill) and white MTA-Angelus, against five different microorganism strains. The materials and their components were evaluated after manipulation, employing the agar diffusion method. A base layer was made using Müller-Hinton agar (MH) and wells were made by removing agar. The materials were placed into the wells immediately after manipulation. The microorganisms used were: Micrococcus luteus (ATCC9341), Staphylococcus aureus (ATCC6538), Pseudomonas aeruginosa (ATCC27853), Candida albicans (ATCC 10231), and Enterococcus faecalis (ATCC 10541). The plates were kept at room temperature for 2 h for prediffusion and then incubated at 37°C for 24 h. The results showed that Sealapex and its base paste, Sealer 26 and its powder, Endo CPM Sealer and its powder, white MTA and its powder all presented antimicrobial activity against all strains. Intrafill and its liquid presented antimicrobial activity against all strains except P aeruginosa and Acroseal was effective only against M. luteus and S. aureus.

Key words: root canal filling material, antimicrobial activity, Mineral Trioxide Aggregate.

INTRODUCTION
The main goal of the root canal filling procedure is to achieve a complete seal of the root canal system. If this step fails, the results of the endodontic therapy may be compromised.

In search of materials with adequate biological properties, the inclusion of calcium hydroxide in the composition of endodontic sealer cements has been proposed.

The first calcium hydroxide-based endodontic cement released was Sealapex. Holland & Souza demonstrated that this material presents excellent biocompatibility when tested in dogs and monkeys and was able to induce apical seal with mineralized tissue.
Other calcium hydroxide-based endodontic cements were later developed. Sealer 26, which combines epoxy resin with calcium hydroxide, has demonstrated good sealing capacity. Acroseal, a sealer with a composition similar to Sealer 26, was more recently released. Studies on pH and calcium release have demonstrated that Sealer 26 promotes less alkalinization of the medium and lower calcium release than other calcium hydroxide-containing cements, such as Sealapex and Acroseal.

Among the newer materials, Mineral Trioxide Aggregate (MTA) has been widely advocated in endodontics for treatment of root perforations, pulp capping, pulpotomy and retrograde fillings. MTA has an alkaline pH and, according to Holland et al., its mechanism of action is similar to that of calcium hydroxide. Recently launched, Endo CPM Sealer is a new MTA-based endodontic cement indicated for root canal sealing. The introduction of endodontic cements containing calcium hydroxide and MTA warrants studies on the antimicrobial effect of these materials and their components, in order to better understand the properties of the cements and to determine which component is responsible for each of the effects observed. The aim of this study was to evaluate the antimicrobial activity of a new root canal sealer containing calcium hydroxide (Acroseal) and the root canal sealer based on MTA (Endo CPM Sealer), in comparison with traditional sealers (Sealapex, Sealer 26 and Intrafill) and white MTA-Angelus, against five different microorganism strains.

**MATERIALS AND METHODS**

The source of the various root canal filling materials is presented in Table 1. The morphotype and source of the strains used as indicators of antimicrobial activity are presented in Table 2. The materials were evaluated in duplicate for antimicrobial activity using the agar diffusion method. The well method was conducted on double-layered plates. The base layer was composed of 10.0 mL sterilized Müller-Hinton agar (MH; Difco, Detroit, MI, USA) poured in 20 X 100 mm sterilized Petri plates. After solidification, a 5.0 mL seed layer, obtained by the addition of the inoculum at a concentration of 10^6 colony forming units/mL to 5.0 mL of MH, was added. Thereafter, eight wells, 4 mm in diameter (one for each material), were made by removal of agar at equidistant points and then filled immediately with the materials to be evaluated. The root canal sealers were manipulated according to the manufacturer’s instructions. The plates were maintained at room temperature for 2 h for prediffusion of the materials, and then incubated at 37°C for 24 h. After solidification, they were incubated at 37°C for 30 min. The inhibition zones around the well were measured. The inhibition zones were calculated as the mean of the diameters of the inhibition zones on each pair of plates.
Statistical analysis was not performed due to the different degrees of diffusion in agar of the various materials/cements. In this context, the area of the inhibition zone does not necessarily reflect the strength of the antimicrobial agent. Thus, the antimicrobial activity of each material was assessed by the presence (or absence) of an inhibition zone, as reported in previous studies\textsuperscript{15-17}. The microorganisms utilized in this study included facultative bacteria and a yeast. These microorganisms are predominant in persistent or refractory periapical lesions\textsuperscript{18,19}.

Our results showed that Sealapex and its base paste, Sealer 26 and its powder, Endo CPM Sealer and its powder, white MTA and its powder presented antimicrobial activity against all strains. The antimicrobial activity of calcium hydroxide-based materials such as Sealapex and Sealer 26 may be related to ionization with subsequent release of hydroxide ions and rise in pH levels, resulting in an unfavorable environment for microbial growth\textsuperscript{4,15}. This effect may be confirmed by the antimicrobial action of both the base paste of Sealapex and the powder of Sealer 26, which include calcium oxide or calcium hydroxide in their composition. Leonardo et al.\textsuperscript{15}, in a study with a similar agar diffusion methodology, observed that Sealapex and calcium hydroxide-based pastes presented antimicrobial action.

The antimicrobial activity of MTA was evaluated by Torabinejad et al.\textsuperscript{20}, who detected its effectiveness against some facultative bacteria. MTA-based materials contain calcium oxide in their composition. When this substance is mixed with water, formation of calcium hydroxide occurs, inducing a rise in pH levels by dissociation of the calcium and hydroxide ions, as demonstrated by Duarte et al.\textsuperscript{10}. Antimicrobial effect was also observed for MTA powder and Endo CPM Sealer, which contain calcium oxide in their formulation, confirming that

### Table 3: Means of the inhibition zones (in millimeters)*

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>M. luteus</th>
<th>S. aureus</th>
<th>P. aeruginosa</th>
<th>C. albicans</th>
<th>E. faecalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sealapex</td>
<td>12</td>
<td>12</td>
<td>10</td>
<td>14.5</td>
<td>10.5</td>
</tr>
<tr>
<td>Sealapex Base</td>
<td>16.5</td>
<td>16.5</td>
<td>12</td>
<td>17.5</td>
<td>10.5</td>
</tr>
<tr>
<td>Sealapex Catalizer</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acroseal</td>
<td>10</td>
<td>13.5</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Acroseal paste A</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acroseal paste B</td>
<td>12.5</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>6.5</td>
</tr>
<tr>
<td>Sealer 26</td>
<td>17.5</td>
<td>18</td>
<td>12.5</td>
<td>9</td>
<td>10.5</td>
</tr>
<tr>
<td>Sealer 26 powder</td>
<td>14</td>
<td>11</td>
<td>10.5</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>Sealer 26 resin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CPM Sealer</td>
<td>20.5</td>
<td>19.5</td>
<td>15</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>CPM Sealer powder</td>
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<td>10</td>
<td>16</td>
<td>8.5</td>
<td>9</td>
</tr>
<tr>
<td>CPM Sealer liquid</td>
<td>10</td>
<td>7.5</td>
<td>6.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MTA (white)</td>
<td>17</td>
<td>16</td>
<td>14.5</td>
<td>14</td>
<td>13.5</td>
</tr>
<tr>
<td>MTA (white) powder</td>
<td>12</td>
<td>12.5</td>
<td>13</td>
<td>14</td>
<td>13.5</td>
</tr>
<tr>
<td>MTA (white) liquid</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Intrafill</td>
<td>22.3</td>
<td>13.5</td>
<td>0</td>
<td>18.5</td>
<td>7</td>
</tr>
<tr>
<td>Intrafill powder</td>
<td>23</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Intrafill liquid</td>
<td>23.5</td>
<td>18</td>
<td>0</td>
<td>25</td>
<td>11.5</td>
</tr>
</tbody>
</table>

*Means of the duplicate assays
calcium oxide plays a role in the antimicrobial activity of these materials. Therefore, the antimicrobial activity of MTA and Endo CPM Sealer may be associated to their elevated pH. Torabinejad et al.\textsuperscript{20} observed an initial pH of 10.2 for MTA, rising to 12.5 in 3 h. It is known that pH levels in the order of 12.0 can inhibit most microorganisms, including resistant bacteria such as \textit{Enterococcus faecalis}.\textsuperscript{21}

In this study, MTA and Endo CPM Sealer presented similar antimicrobial activity, suggesting that the changes in composition during the manufacturing of Endo CPM Sealer did not interfere with the antimicrobial action of MTA.

Intrafill and its liquid presented antimicrobial activity against all strains except \textit{P. aeruginosa}, and Acroseal was effective only against \textit{M. luteus} and \textit{S. aureus}. The antimicrobial action of the zinc oxide and eugenol-based cements such as Intrafill is due to the presence of eugenol. Saleh et al.\textsuperscript{22} evaluated the survival of \textit{E. faecalis} in infected dentin tubules, after sealing the root canals with different endodontic cements. Sealing the canals with gutta-percha and AH Plus or Grossman cement (zinc oxide and eugenol-based) was more effective in eliminating \textit{E. faecalis} than sealing with Roekoseal. Using a similar protocol, Sipert et al.\textsuperscript{16} found “in vitro” antimicrobial action for Sealapex, Fill Canal, Pro Root MTA, and Portland. Fill Canal has a similar composition to Intrafill, which did not show antimicrobial activity against \textit{Pseudomonas aeruginosa} in the present study.

Tanomaru-Filho et al.\textsuperscript{23} observed similar antimicrobial activity in MTA-based Portland cement, and other endodontic materials used in retrograde obturation, such as Sealapex with zinc oxide and Sealer 26. The results of this study revealed even lower antimicrobial action for Acroseal cement. This material, despite presenting a formulation comparable to Sealer 26, did not show the same antimicrobial activity. Its effect was restricted to inhibiting \textit{M. luteus} and \textit{S. aureus}.

\textbf{REFERENCES}

13. Georgopoulou M, Kontakiotis E, Nakou M. In vitro evaluation of the effectiveness of calcium hydroxide and