Evaluation of the Antimicrobial and Cytotoxic Activity of Epiphany Root Canal Sealer in Vitro

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This study evaluated the antimicrobial and cytotoxic effect of Epiphany root canal sealer versus commonly used sealers; EndoFill, Apexit, Ketac-Endo, and AH-26. The first portion of this study used four different microorganisms; Enterococcus faecalis (E.faecalis), staphylococcus aureus (S. aureus), Eschericia coli (E.coli) and Candida albican (C. albican) to determine the antimicrobial effect using the agar-well diffusion method. Secondly, In vitro Cytotoxicity assay using human periodontal ligament cell culture was used to evaluate the cytotoxicity of Epiphany sealer compared to the other four sealers. The cell cultures were incubated for 24h with freshly mixed material and after 24 h of setting. The viability of the fibroblast cells was determined using trypan blue. The results of this comparative study indicated that Epiphany sealer exhibited a potential antibacterial activity with no anticandida effect. The cytotoxicity of Epiphany was comparable to that of EndoFill and Apexit, however it was significantly less toxic than Ketac-Endo, and AH-26.

INTRODUCTION

The role of bacteria and their by-products in the initiation and perpetuation of pulp and periapical diseases has been well established. Most infecting bacteria, together with their principal substrate of necrotic pulp debris, may be removed by routine endodontic procedures such as instrumentation, irrigation, and the use of an intracanal medicament with antimicrobial activity1. However, infection may persist in canals with high anatomic complexity, with a great number of bacteria, especially facultative anaerobic bacteria2. In addition, several studies have associated the presence of fungi with therapy-resistant endodontic infections3.

After the microbial control phase of endodontic therapy, a root canal filling is placed to seal the root canal system from the external environment4. The standard method of obturation of the root canal space is by using a core material in combination with a root canal sealer4. Antimicrobial activity plays an important role in the efficacy of an endodontic sealer during root canal filling, and for this reason many studies have dealt with the antibacterial activity of endodontic sealers5.

Faculative microorganisms such as E. faecalis, E.coli and S.aureus and even C.albicans have been considered to be the most resistant species in the oral cavity and possible cause of failure of root canal treatment6. The agar diffusion method has been widely used to test the antimicrobial activity of dental materials and medications, the advantage of this method is that it allows direct comparisons of root canal sealers against the test microorganisms, indicating which sealer has the potential to eliminate bacteria in the local microenvironment of the root canal system7.

Good tissue compatibility is decisive for root canal sealers because they may come into direct contact with tissues specially when extruded to the apical area8. Toxicity testing of dental materials can be assessed either in vitro or in vivo. Using cell lines is a common method of testing dental materials that allows for a simple, reproducible result that can be controlled in a laboratory setting. In vitro testing also allows for the comparison between several materials using the same cells under the same conditions9.

Improvements in adhesive technology have fostered attempts to reduce apical and coronal leakage by bonding to canal walls10. These improvements rely on the incorporation of resin monomers into the sealer or application of resins during a distinct conditioning step. Other strategies have focused on substitutes for gutta percha that bond to the root dentin, thereby establishing a so called monoblock-obturation11.
The Epiphany obturating system (Pentron Clinical Technologies, Wallingford, CT) which is a dual curable dental resin composite sealer uses Resilon points and is bonded to the root dentin. Resilon (Resilon Research LLC, Madison, CT), a thermoplastic synthetic polymer based root canal filling material, has been developed that performs like gutta-percha, has the same handling properties, and for retreatment purposes may be softened with heat or dissolved with solvents like chloroform. Based on polymers of polyester, Resilon contains bioactive glass, bismuth oxychloride and barium sulfate. Resilon associated with Epiphany has recently been introduced. Some of its mechanical and chemical properties have been evaluated with controversial results. In contrast, the biological properties of Resilon and Epiphany are not well documented.

The properties of root canal cements can be divided into physicochemical, antimicrobial and biological. Therefore, the aim of this study was to in vitro evaluate the antimicrobial and cytotoxic properties of Epiphany sealer in comparison with the commonly used root canal sealers.

MATERIALS AND METHODS

The various root canal sealers that were tested in the present study are the adhesive resin root canal sealer (Epiphany), ZOE based sealer (EndoFill), calcium hydroxide based sealer (Apexit), glass ionomer based sealer (Ketac-Endo) and Resin sealer (AH-26).

Antimicrobial test
Agar-well diffusion method (AWDM):
Cultures of E. faecalis, S. aureus, E.coli and C. albican were obtained from endodontic isolates. All microorganisms were previously subcultured in appropriate culture plates and under gaseous conditions to confirm their purity. From the broth culture suspensions of the tested microorganism 0.2 ml were prepared adjusted to No. 0.5 McFarland scale were spread on four Petri dishes containing Mueller-Hinton Agar medium. Five wells of 5mm depth and 4mm diameter were punched in the agar plates and filled with the test sealers (mixed according to manufacturer instructions). The plates were incubated aerobically at 37°C for 48h. The diameters of the zones of microbial inhibition around each well were measured in millimeters after the incubation period. The inhibitory zone was considered to be the shortest diameter from the outer margin of the well to the initial point of the microbial growth.

Three replicates were measured for each microorganism and a mean diameter was determined for each sealer. Greater diameters of zones of inhibition were interpreted to indicate greater antimicrobial activity of the involved sealers.

Cytotoxicity assay
Test materials and specimen preparation:
The tested materials were mixed according to the manufacturer's instructions. Freshly mixed materials were filled in polyethylene rings of 5mm inside diameter and 4mm in height. The test was performed on fresh mix and 24h set mix. To prevent contamination of the set samples they were exposed to UV light for 2h before performing the test. In a pilot study made previously, this period was sufficient to sterilize experimental materials.

Cell Culture:
Periodontal ligament cells (PDL) were obtained from teeth extracted for orthodontic purposes. Fibroblasts were grown in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal bovine serum and antibiotics (10,000 units of penicillin-G/ml, 10 mg of streptomycin/ml and 20 mM of L-glutamine).

Growth Measurement:
The fibroblast cells were plated at a density of 3-4 x10⁶ cells/ml and dispensed onto 96-well culture plate with 1ml of medium per well and incubated at 37°C supplemented with 5% CO₂ for 48h to allow
attachment of the fibroblasts to the bottom of the wells. Specimens of different sealers were placed onto the wells of the culture plate and each specimen was covered by 100µl suspension of fibroblasts and incubated at 37°C with 5% CO₂ for 48h. At the end of the incubation period, the culture medium was aspirated and 100µl of formaldehyde phosphate buffered saline (FPBS) was added to each culture well for 2h. FPBS was then removed and each well was thoroughly rinsed with distilled water and 100µl of 0.25% of trypan blue (wt/vol) was added to each well to stain the nonviable cells. After 3h, the trypan blue was removed and the cells were thoroughly rinsed with distilled water. The number of viable cells was expressed by averaging of six readings using an inverted microscope (Olympus, 1X71, Japan). Statistical analysis was performed by one way ANOVA, and Bonferroni tests.

RESULTS

Table 1 shows the mean of the zones of inhibition of microbial growth of each sealer against the microorganisms tested measured in mm and the average values of the antimicrobial activity of each sealer against all microorganisms. EndoFill produced the largest inhibitory zone followed by Epiphany (by average values). On the other hand, Apexit produced the smallest inhibitory zones against the tested microorganisms (by average values). It appears that Candida albican was the most resistant organism to the effect of the sealers in this experiment. Epiphany had the largest inhibitory zone on E. faecalis followed by S. aureus and E. coli. However, Epiphany had no effect on C. albican. Figures 1-4 show the zones of inhibition of the tested sealers with the different microorganisms.

Table 1: Mean values of antimicrobial activity of root canal sealers against microorganisms tested (mm):

<table>
<thead>
<tr>
<th></th>
<th>Epiphany</th>
<th>EndoFill</th>
<th>Apexit</th>
<th>Ketac-Endo</th>
<th>AH-26</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. faecalis</td>
<td>9</td>
<td>7</td>
<td>1.3</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>S. aureus</td>
<td>6</td>
<td>9</td>
<td>5</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>E. coli</td>
<td>5</td>
<td>6</td>
<td>0.66</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>C. albicans</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Average inhibition zone of sealers for all microorganisms</td>
<td>5</td>
<td>7.25</td>
<td>1.74</td>
<td>2.75</td>
<td>3.75</td>
</tr>
</tbody>
</table>
Fig. 1: Inhibition zones of the tested sealers with *E. faecalis*.

Fig. 2: Inhibition zones of the tested sealers with *S. aureus*.

Fig. 3: Inhibition zones of the tested sealers with *E. coli*.

Fig. 4: Inhibition zones of the tested sealers with *C. albican*.

1) Epiphany  2) EndoFill  3) Apexit  4) Ketac-Endo  5) AH-26
Table 2: Mean and standard deviation of viable cells after exposure to different sealers at 0-h (fresh) and 24-h.

<table>
<thead>
<tr>
<th>Material</th>
<th>0-h</th>
<th>SD</th>
<th>24-h</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epiphany</td>
<td>28.1a</td>
<td>0.4</td>
<td>30.5 a</td>
<td>0.83</td>
</tr>
<tr>
<td>EndoFill</td>
<td>27.3a</td>
<td>1.36</td>
<td>29.3 a</td>
<td>1.36</td>
</tr>
<tr>
<td>Apex</td>
<td>29.8a</td>
<td>2.71</td>
<td>32.6 a</td>
<td>1.63</td>
</tr>
<tr>
<td>Ketac-Endo</td>
<td>18.8c</td>
<td>0.752</td>
<td>22.3 c</td>
<td>3.26</td>
</tr>
<tr>
<td>AH-26</td>
<td>19.3 c</td>
<td>1.21</td>
<td>25.6 c</td>
<td>0.516</td>
</tr>
</tbody>
</table>

Values are means ± SD. Different superscript letters (a-c) indicate groups that are significantly different (p≤0.05). Groups identified with the same superscript letters are not significantly different (p>0.05).

Fig. 5: Histogram representing the Percentage of fibroblast cells viability after exposure to different sealers.
The results of cytotoxic effect evaluation of the examined sealers on fibroblast cells are shown in Table 2 and Figure 5. Although every material tested showed some degree of toxicity, the percentage of cell viability was greater with Epiphany, EndoFill and Apexit than with other sealers with no statistically significant difference between these three sealers in both time periods (fresh and 24 h). Other materials (Ketac-Endo, AH-26) showed significantly lower percentage of cell viability. All sealers showed increased number of viable cells after 24 h.

**DISCUSSION**

A number of approaches have been used to evaluate the antimicrobial effectiveness in the laboratory. These include; incubation of broth cultures of the selected microorganisms with the agent under the test, growth of the selected microorganisms as lawns on agar surfaces using the disc diffusion method, and the artificial infection of extracted teeth with the selected organisms and irrigation with the test agent 21.

The agar diffusion method used in this study is one of the most often used methods for the antimicrobial activity assessment. The results of this method do not depend only on the toxicity of the material for a particular microorganism, but also on the diffusibility of the material across the medium. However, great care has been taken in this study to keep the plates for 2h at room temperature to allow the diffusion of the agent through the agar and then incubated at 37ºC under appropriate gaseous conditions. The present study tested the antimicrobial activity of different sealers against microorganisms considered to be resistant to endodontic treatment. Therefore, if a sealer is effective against these microorganisms, it will probably be effective against the most susceptible ones 22.

In this study, Epiphany produced a clear antibacterial effect against the tested bacteria especially the most resistant one (*E. faecalis*). On the contrary, Bodrumlu and Semiz 23 showed that Epiphany root canal sealers had little effect on *E. faecalis*. This can be explained by a research conducted by Gomes et al. 5, who determined that the antimicrobial activity depends on the sensitivity of the drug, bacterial source (wild strains or collection species), number of bacteria inoculated, pH of the substrates in plates or tubes, agar viscosity, storage conditions of the agar plates, incubation time and the metabolic activity of the microorganisms.

The results of this study showed that Epiphany had no effect on *C.albicans*. Moderate inhibition was produced by Epiphany on *E.coli* and *S. aureus* isolates.

EndoFill had the maximum average zones of inhibition as compared to other tested sealers. However, it comes next to Epiphany regarding the effect against *E.faecalis*. On the other hand, this material was the only effective sealer on *C.albican*. Previous studies indicated that root canal sealers containing ZOE have a strong antibacterial effect 24. This effect was due to the action of eugenol 25. Kaplan and others have stated that the most effective antimicrobial sealers contain eugenol and formaldehyde 26.

Other endodontic sealers tested (Apexit, AH-26 and Ketac-Endo) were less effective than Epiphany in killing the microorganisms. The antimicrobial activity of Apexit may be based on its content of calcium hydroxide. Root canal sealers integrated with calcium hydroxide have enhanced antimicrobial activity 23, 27. The antimicrobial effect of this sealer is produced by the release of hydroxyl ions which increases the pH above 12.5 23. In this experiment Apexit had the lowest average diameter of inhibition zones. Bystrom and Sundqvist 28 also found that for a calcium hydroxide based sealer to be an effective antimicrobial agent, it should maintain a pH level greater than 12.5. As the calcium hydroxide sealer sets, the pH declines rapidly to 9.4 causing loss of effectiveness.

Some endodontic sealers consists of polymer materials such as AH-26 which release small amount of formaldehyde during the polymerization process, and it is this agent that gives the resin based sealer its antimicrobial effect 29. Bodrumlu and Semiz 23 showed that AH-26 had the lowest antibacterial effect against *E.faecalis*, and attributed this effect to the release of a small amount of formaldehyde over a brief period of time. In this study AH-26 had a minor effect against the tested organisms and in
particular, it had a moderate effect on E. faecalis.

The antimicrobial activity of Ketac-Endo is assumed to rely on fluoride ion release, but the quantities of fluoride released may not be sufficient to reach a concentration that effectively kills microorganisms 30. A weak antimicrobial activity of Ketac-Endo has been reported by Heling and Chandler 31.

Antimicrobial activity of root canal sealers helps destroy the remaining bacteria. On the other hand, severe toxicity of a filling material may be a reason for damage of the periapical tissue thereby abolishing the beneficial effects of the antimicrobial properties of the material 22. The antimicrobial components of the sealer do not have selective toxicity against microorganisms; they also exert toxic effects on host cells 31. Azar, et al 32 thought that in general, the toxicity of newly developed materials should be assessed using the three steps approach. A first step is to screen a candidate material using a series of in vitro cytotoxicity assays using cell cultures. Then, if the material is determined not to be cytotoxic in vitro, it can be implanted in subcutaneous tissue or muscle and the local tissue reaction evaluated. Finally, the in vivo reaction of the target tissue versus the test material must be evaluated in human subjects or animals. Therefore, if the test material induces a strong cytotoxic reaction in cell culture tests, it is very likely also to exert toxicity in living tissue. A reduction in the number of animal tests and the resulting expenses might be an additional benefit of such a screening approach.

In this study, the cytotoxicity was evaluated by measuring the cell growth via cell counting. This method was chosen because it is easy, not requiring complicated or expensive equipments.

In this present study, an in vitro cytotoxicity tests was done by using primary cells, mainly oral fibroblasts, to test the dental materials. Primary cell lines have a predetermined life span and will eventually reach a plateau of growth and then die even if the conditions for growth are acceptable. Because the materials tested would more likely come into contact with human fibroblasts in vivo, they were chosen to more closely represent clinical conditions 33.

This study showed that the Epiphany root canal sealer was less toxic than AH-26 and Ketac-Endo, the values of the cytotoxicity of Epiphany were not significant when compared to EndoFill and Apexit. These results are in agreement with Souza et al 34 who showed that Epiphany root canal sealer was the only material that presented intraosseous biocompatibility compared to AH Plus and EndoRez.

On the contrary, Susini 14 et al showed that Epiphany + Resilon were the most cytotoxic material at 1 and 2 days and this toxicity decreases with time until become nontoxic at 7 days. They explained this toxicity to be due to leaching of uncured monomers from the bulk of the resin because Epiphany set under anaerobic conditions and no curing system leads to a 100% of conversion 35.

The results of this study concerning the ZOE sealer were similar to previous findings that showed that this material is moderately toxic and this toxicity is attributed to the release of free eugenol which is the main liquid components of ZOE based sealers 36, 37. It is interesting to know that when zinc oxide eugenolate is formed, most of the free eugenol is bound which account for the relatively low toxicity of ZOE based sealers in the initial setting reaction 38. However, after 48h enough free eugenol escaped to cause the reaction to become predominantly severe 39.

This study indicated that AH-26 had a moderate to severe cytotoxic reaction immediately after mixing and this toxicity decreased after 24h. These effects may be due to formaldehyde, which is released during the initial setting reaction. It has been demonstrated that AH-26 is relatively cytotoxic sealer 38, 40 as it releases formaldehyde during and after setting 39. Periapical inflammatory reactions were detected when AH-26 was used as sealer 42.

Ketac-Endo showed a pronounced cytotoxic effect in the early phase of the setting reaction and this toxicity decreased after 24h. This observation is in agreement with a previous study which showed strong inflammatory reactions caused by Ketac-Endo 40. Schwarze 36 et al. explained this toxicity by the high water solubility of the glass ionomer cement in the early phase of the setting
reaction leading to the elution of cytotoxic substances.

**In conclusion**, Epiphany sealer demonstrates a clear antibacterial activity, particularly against *E. faecalis* which is one of the most resistant endodontic flora. However it had no activity against fungi (*C. albicans*), so the emergence of yeast with resistance to Epiphany sealer warrants continued, prudent monitoring.

The cytotoxicity of Epiphany was comparable to that of EndoFill and Apexit, on the other hand it was less toxic than Ketac-Endo and AH-26.

Further in vivo studies are needed to confirm the value of Epiphany sealer using a root model.

**REFERENCES**


