An in vitro evaluation of antifungal activity of bioaggregate

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Objective. The aim of this study was to evaluate, in vitro, the antifungal effect of bioaggregate (BA) against Candida albicans by using the direct contact method.

Study design. BA was tested freshly mixed and after 24-hour set on C. albicans. The tested BA was incubated with C. albicans in plastic tissue clusters for 1 hour, 24 hours, and 3 and 5 days. Aliquots of 0.1 mL were taken from each well at the end of the incubation periods and transfered to tubes containing 5 mL fresh Sabouraud broth. All tubes were vortexed and then incubated at 37°C and observed for the subsequent 5 days. Growth of the fungi was observed daily by the presence of turbidity in the tubes. The results were statistically analyzed by using Kaplan-Meier test.

Results. The freshly mixed and set BA had no antifungal effect at 1 and 24 hours of contact. Both mixes demonstrated complete fungicidal activity after 24 hours’ contact. Statistical analysis showed a highly significant difference between the negative and positive control groups (P<.001) and a significant difference between the freshly mixed and 24-hour set BA groups (P<.001) at 24 hours.

Conclusions. BA (freshly mixed and 24-hour set) was effective against C. albicans after 24 hours.

Bioaggregate (BA), laboratory-synthesized water-based cement, was recently developed aiming for the improvement of some properties of the well studied mineral trioxide aggregate (MTA) cement. It has the same indications for use as MTA, including vital pulp therapy, perforation repair, retrograde filling, and apexification, which has proved to be the gold standard of all materials used in surgical and nonsurgical endodontic treatment. In addition, BA has been reported to display in vitro compatibility similar to MTA1,2 as well as antimicrobial activity against Enterococcus faecalis.3 The antifungal properties of MTA have been evaluated by several investigators.4-8 They all reported good fungicidal effect against Candida albicans. However, the antifungal activity of BA was not yet been reported. Therefore, the aim of the present study was to evaluate, in vitro, the antifungal activity of BA by using the direct contact method.

MATERIAL AND METHODS

The effect of the antifungal activity of the BA (Innovative Biocaramix, Vancouver, Canada) was evaluated (freshly prepared and after 24-hour set) against Candida albicans.

Stock cultures of clinically isolated C. albicans (strain no. 66027) provided by the Microbiology Laboratory of King Khalid University Hospital (King Saud University, Riyadh, Saudi Arabia) were maintained in Sabouraud agar plate. A suspension was prepared by transferring 3 colonies from the Sabouraud agar plate by using a sterile 4-mm diameter platinum loop to 10 mm Sabouraud dextrose broth in a sterilized 10 mL screw-capped test tube, followed by incubation for 2 days at 37°C. Six such test tubes were prepared.

Experiment procedure

The experiment was performed in plastic tissue culture clusters (Costar Corning, Corning, NY) containing 24 wells each with an inner diameter of 16 mm. A total of 40 wells were used and divided into 2 experimental groups (freshly mixed BA and 24-hour set BA) and control groups (positive and negative) of 10 wells each. For the BA groups, 1 pack of BA (1 g) was carefully mixed at the bottom of each culture well according to the manufacturer’s instructions. The 24-hour set BA group was placed in the incubator at 37°C for 24 hours after mixing. Afterward, 2 mL Candida suspension
solution was placed into the wells containing the freshly mixed BA as well as the 24-hour set BA. In addition, 1 mL Sabouraud broth media was mixed with 1 mL of Candida suspension in a culture well. This served as positive control. For the negative control test, 2 mL Sabouraud broth was placed in culture well. The culture-cluster plates were then incubated at 37°C and evaluated after 1 hour and 1, 3, and 5 days. At the end of each incubation period, aliquots of 0.1 mL were taken from each well without mixing the content of the well and transferred to tubes containing 5 mL fresh Sabouraud broth. All tubes were vortexed and then incubated at 37°C and observed for the subsequent 5 days.

Growth of the fungi was observed daily by the presence of turbidity in the tubes. The presence of turbidity was determined, and the purity of the culture was checked by morphology of colonies onto Sabouraud agar. The results were statistically analyzed using Kaplan-Meier test at the level of significance \( \alpha = .05 \).

RESULTS

The results are summarized in Tables I-III.

Control

No fungal growth was shown in the negative control samples during the period of examinations, whereas the positive control samples demonstrated entirely fungal growth.

Freshly mixed BA

Fungal growth was observed during 1-hour and 1-day incubation of C. albicans with the freshly prepared BA. Increasing the exposure time to 3 and 5 days of C. albicans to the freshly prepared BA, however, resulted in complete inhibition of growth.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Neg. control</th>
<th>Pos. control</th>
<th>BA fresh mix</th>
<th>BA (24-hr set)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 h 1 d 3 d 5 d</td>
<td>1 h 1 d 3 d 5 d</td>
<td>1 h 1 d 3 d 5 d</td>
<td>1 h 1 d 3 d 5 d</td>
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<tr>
<td>2</td>
<td>— — — —</td>
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<td>* * * *</td>
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<tr>
<td>3</td>
<td>— — — —</td>
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<td>4</td>
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<td>5</td>
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<td>* * * *</td>
<td>* * * *</td>
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<td>6</td>
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<td>7</td>
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</table>

Table I. Evaluation of the effect of the direct contact of tested materials on cultured Candida albicans

Table II. Means and medians for survival time

<table>
<thead>
<tr>
<th>Bioaggregate</th>
<th>Estimate</th>
<th>SE</th>
<th>Lower bound</th>
<th>Upper bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>C−</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>C+</td>
<td>120.000</td>
<td>0.000</td>
<td>120.000</td>
<td>120.000</td>
</tr>
<tr>
<td>BA fresh mix</td>
<td>72.000</td>
<td>0.000</td>
<td>72.000</td>
<td>72.000</td>
</tr>
<tr>
<td>BA 24-h set</td>
<td>24.000</td>
<td>0.000</td>
<td>24.000</td>
<td>24.000</td>
</tr>
<tr>
<td>Overall</td>
<td>54.000</td>
<td>7.380</td>
<td>39.536</td>
<td>68.464</td>
</tr>
</tbody>
</table>

C−, Negative control; C+, positive control; BA, bioaggregate. *Estimate is limited to the largest survival time if it is censored.

Table III. Overall comparisons

| Test of equality of survival distributions for the different levels of bioaggregate. |
|------------------------------|-------------------|-----------------|-------------------|
| Log rank (Mantel-Cox)        | 62.086            | 3               | .000              |
| Breslow (generalized Wilcoxon)| 53.886            | 3               | .000              |
| Tarone-Ware                  | 57.746            | 3               | .000              |

24-hour set BA

Fungal growth was observed during 1-hour and 1-day incubation of C. albicans with the set BA. When the incubation period increased to 3 and 5 days, no fungal growth was observed.

Statistical analysis showed a highly significant difference between the negative and positive control groups (\( P = .000 \)) and between the freshly mixed and 24-hour set BA groups (\( P = .000 \)) after 24 hours’ observations.
DISCUSSION

The method used in this study has an advantageous in allowing direct contact between fungi and the material in solution.\(^9\) In addition, it minimizes possible confounding factors in the experiment.\(^3\) Such advantages explained the rationale of its methodologic choice in a previous study.\(^4\) The effectiveness of this method was confirmed by the observation of the positive control samples.

A great deal of scientific evidence indicates that microorganisms involved in intraradicular or extraradicular infections are the major causative agents of endodontic therapy failure, including fungi.\(^10,13\) *Candida albicans* has been reported to be the most commonly isolated fungal species. Siqueira and Sen reported that *C. albicans* is able to colonize root canal walls and penetrate into dental tubules.\(^14\) Grossman\(^15\) reported that the presence of *Candida* organisms in infected root canals causes a real problem in endodontic treatment. He insisted in eliminating these organisms for better prognosis. *Candida albicans* was found to be more resistant than *E. faecalis* or *Bacillus* species when evaluating the antimicrobial effects of citric acid and sodium hypochlorite.\(^16\) In addition, Sen et al.\(^17\) reported that the antifungal properties of 0.12% chlorhexidine, 1% NaOCl, and 5% NaOCl was affected by the presence of smear layer. They found *C. albicans* to be more resistant in the presence than in the absence of smear layer. Furthermore, *C. albicans* cells were reported to be highly resistant to calcium hydroxide.\(^18\) Both MTA and BA produce calcium hydroxide by a hydration reaction. Siqueira et al.\(^19\) reported that even in a harsh calcium hydroxide environment, it took 1 week to totally eliminate *C. albicans* and 2 days’ exposure to disinfect most of a specimen. A mixture of 2% chlorhexidine and calcium hydroxide was found to be a very effective antifungal agent against *C. albicans*.\(^20\) The involvement of fungi in cases of persistent and secondary infections associated with recalcitrant periradicular lesions require the use of intracanal medicament and repaired filling material with antifungal activity.

The present study has demonstrated that BA, both freshly mixed and 24-hour set, is effective in killing *C. albicans* at 3 and 5 days’ observation. The material did not show antifungal activity at 1 hour and 24 hours’ observation. A clear explanation of such delayed antifungal activity is still unknown. An earlier study demonstrated that pH of BA peaked at 24 hours, which could explain the delayed activity.\(^3\) In addition, *C. albicans* has been reported to be more resistant to high pH in vitro than other persisting microorganisms, such as *E. faecalis*.\(^18,21,22\) Zhang et al.\(^3\) and Al-Nazhan and Al-Juda\(^4\) reported that the release of diffusible substances into the growth media of MTA and BA is primarily responsible for killing *C. albicans*.

Tantalum oxide is the major difference between MTA and BA. A significant amount of tantalum oxide is present in the BA material. It has been used as sutures, plates, and membranes in orthopedics because of its inertness.\(^23,24\) A strong inhibition zone when osteoblasts were grown has been reported by Steinmann, whereas fibroblasts proliferated well on the tantalum disk.\(^25\) The antimicrobial activity of tantalum oxide was reported by Pratt and Smith.\(^26\) Thus, the presence of tantalum oxide could play a role in the antifungal effectiveness of the BA material.

In a previous study, we found that MTA (freshly mixed and 24-hour set) was effective in killing *C. albicans* after 24 hours of contact.\(^4\) In the present study, BA displayed in vitro antifungal activity similar to white MTA.

In conclusion, BA (freshly mixed and 24-hour set) displayed an in vitro effect on the tested *C. albicans* after 24 hours of contact.

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REFERENCES


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