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Evaluation of Antifungal Activity of Mineral Trioxide Aggregate

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The purpose of this investigation was to study, in vitro, the antifungal effect of mineral trioxide aggregate (MTA) using a tube-dilution test. MTA was tested freshly mixed and after 24 h set on *Candida albicans.* The tested MTA was incubated with *C. albicans* for 1 h, 24 h, and 3 days. Results showed that the freshly mixed MTA was effective in killing the tested fungi after 1 day of contact, whereas the 24-h set MTA was effective after 3 days of incubation. It was concluded that MTA (freshly mixed and 24-h set) was effective against *C. albicans.*

An experimental material, mineral trioxide aggregate (MTA), has been developed recently to seal pathways of communication between the root-canal system and the external surface of the tooth. This material has been investigated extensively by Torabinejad et al. (1–3) and was reported to be a promising material. It was suggested for different pulpal and endodontic therapy (4).

The antimicrobial effects of MTA materials have been studied (5). It has an antibacterial effect on some of the tested facultative bacteria. However, their antifungal activity was not yet reported. The purpose of this investigation was to study, in vitro, the antifungal activity of MTA using a tube-dilution test.

MATERIALS AND METHODS

The effect of the antifungal activity of MTA (Dentsply, Tulsa, OK, batch #990903) was evaluated (freshly prepared and after 24 h set) against *Candida albicans*. Stock cultures of clinically isolated *C. albicans* provided by the Microbiology Laboratory of King Khalid University Hospital (Riyadh, Saudi Arabia) were maintained in Sabouraud agar plate. A suspension was prepared by transferring three colonies from the Sabouraud agar plate using a sterile 4-mm diameter platinum loop to 10 mm of Sabouraud infusion broth in a sterilized 10 ml screw-capped test tube and then incubated for 1 week at 37°C. Two test tubes were prepared.

Experimental Procedure

The experiment was performed in plastic tissue-culture clusters containing 24 wells each with an inner diameter of 16 mm. One pack of MTA was mixed at the bottom of each culture well. Afterward, 2 ml of the *Candida* suspension solution was placed into the wells containing MTA. In addition, 1 ml of Sabouraud infusion-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 of Sabouraud infusion broth was placed in culture well. Six wells were used per test. The culture-cluster plates were then incubated at 37°C and evaluated after 1 h, 1 day, and 3 days. At the end of each incubation period, aliquots of 0.1 ml were taken from each well and transferred to tubes containing 5 ml of fresh Sabouraud infusion broth. All tubes were vortexed and then incubated at 37°C and observed for the consecutive 7 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 cultures was checked by morphology of colonies onto blood-agar plates. The results were statistically analyzed using Kruskal-Wallis test.

RESULTS

Control

The negative control showed no fungal growth in all the experimental period of observations, whereas the positive control demonstrated entirely fungal growth.

MTA Freshly Mixed

Fungal growth was observed during the 1-h incubation of *C. albicans* with the MTA. When the incubation time increased there was no growth in the 1 and 3 days of observation.

24-h Set MTA

Fungal growth was observed during the 1-h incubation of *C. albicans* with the set MTA. When the incubation period increased for 1 and 3 days, no fungal growth was observed.

Statistical analysis showed a highly significant difference between the negative and positive control groups ($p < 0.001$) and no significant difference between the freshly mix and 24-h set MTA groups ($p > 0.05$) in all observation periods.

DISCUSSION

The method used in this study is the dilution–tube-susceptibility test, which is an effective method to evaluate the antifungal and antibacterial properties of any filling material or solution (6). This method allows direct contact in the solution between fungal cells and the MTA material. In addition, such method is considered appropriate when evaluating antifungal activity of MTA, which has a low solubility and diffusibility (2).

C. albicans was chosen as a test organism in this study. It has been found in the infected root canal and in the periradicular tissue (7–10). It is more commonly isolated from persistent infections with apical periodontitis (8, 11, 12). They may enter the pulp through dentinal tubules, deep caries lesion, and fracture, or as contaminants from the oral microflora during root-canal treatment (11, 13).

The results of this experiment showed that freshly mixed and 24-h set MTA is effective in killing *C. albicans* in the 1 and 3 days observation. Torabinejad et al. (2) reported that MTA has long setting time of 3 h. During this time, a chemical reaction of the mixed material is still taking place where the mixed elements might be not effective. In addition, *C. albicans* might be resisting the MTA materials for only short period of time. This could explain the positive growth of the *C. albicans* in both fresh and 24-h set experiment during the 1-h observation.

The antifungal effect of MTA against *C. albicans* was caused by its high pH (2) or release of diffusible substances into the growth media. According to Torabinejad et al. (2), MTA has a pH of 10.2 initially, which rises to 12.5 3 h after mixing. This is similar to calcium-hydroxide material. Waltimo et al. (14) reported that *C. albicans* survived incubation in calcium hydroxide solution for 1 and 6 h and was killed after 6 h of incubation. Similar findings were noticed in this study. The 1-h observation showed positive growth of the *C. albicans* both in fresh and 24-h set MTA.

Based on the results of this investigation, it seems that MTA has good antifungal effect on the tested *C. albicans.*

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