Analysis of the Effects of Calcium Hydroxide, Chlorhexidine and Mineral Trioxide Aggregate on the Viability of *Candida albicans*

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**Abstract**

**Objective:** The aim of this study was to evaluate the effects of calcium hydroxide, chlorhexidine, and mineral trioxide aggregate (MTA) on the viability of *Candida albicans*.

**Method:** Sabouraud Dextrose Agar plates were prepared that contained different concentrations of calcium hydroxide, chlorhexidine, or MTA powder. The plates were inoculated with an overnight culture of *C. albicans*, and the presence of colonies that formed were observed after incubation at 37°C for 1, 24, 48, and 72 hours.

**Results:** Chlorhexidine and MTA, but not calcium hydroxide, inhibited colony formation. The minimum inhibitory concentration of MTA and chlorhexidine against *C. albicans* was 50 mg/ml.

**Conclusions:** We found that MTA and chlorhexidine inhibited the growth in agar of *C. albicans* within three days.

**Keywords:** Antifungal effect; *Candida albicans*; chlorhexidine

**Introduction**

The aim of root canal treatment is to eliminate of microorganisms and to prevent re-infection, because bacterial infection of the root canal is the primary cause of apical periodontitis [1,2]. Further, infected root canals harbor a diverse microbial flora. For example, fungi can be isolated from 5-20% of patients with infected root canals [3]. Unfortunately, fungal infections do not respond well to conventional root-canal therapy [4,5]. *Candida albicans* is among the most commonly isolated fungal species in the oral cavity [6]. Local and systemic antibiotics as well as prolonged endodontic treatment can favour the colonization of the root canal by *C. albicans*.

Current endodontic agents should demonstrate biocompatibility and high antimicrobial properties to eliminate microorganisms from the root canal. For example, calcium hydroxide possesses antimicrobial activity and creates a hard tissue barrier, making it a good choice as an intercanal medication. Its high pH destroys the bacterial cell membrane and denatures proteins due to the release of hydroxyl ions [7]. Chlorhexidine is an effective endodontic disinfectant that is used as intracanal medication because of its ability to penetrate deep into dentin tubules and destroy pathogenic bacteria [8]. Chlorhexidine molecules are positively charged and strongly attracted to negatively charged ions present on the surface of bacteria [7]. Because of its biological and histological properties, mineral trioxide aggregate (MTA) is used for root canal repair and retrograde filling as well as creating a hard tissue barrier during apexification and pulp perforation [9]. Therefore, the aim of this study was to evaluate the effects of calcium hydroxide, chlorhexidine, and MTA on the viability of *C. albicans in vitro* and to determine the minimal inhibitory concentration (MIC) of these agents.

**Material and Methods**

Five plates for each concentration of Sabouraud Dextrose Agar (Liofilchem, Italy) agar were prepared by mixing calcium hydroxide, 2% chlorhexidine (Hibbios, Bosnalijek, BiH), and MTA powder (ProRoot MTA, Dentsply, Tulsa Dental, OK, USA) diluted with 10 liquified agar (45°C) so that the concentration of each compound ranged from 50 mg/ml-0.78 mg/ml (being halved each time), (Figure 1).

As positive control groups served five plates of sabouraud dextrose agar without tested material, and as negative control groups served five plates without *Candida albicans*. The *C. albicans* (ATCC 10231, Liofilchem, Italy) inoculum (10-µl per plate) was prepared by growing an overnight culture from a stock culture. The plates were placed in an incubator (Innovens 53, Jouan, France) set to 37°C, and the colonies on each plate were observed after 1, 24, 48, and 72 hours. The statistical significance of the differences in colony presence among the experimental and control plates were evaluated using the Kruskal-Wallis test. Statistical significance was defined as p<0.05.

**Results**

Compared with the untreated control, *C. albicans* colonies were not
present in either 50 mg/ml each of calcium hydroxide or chlorhexidine during the first 24 h. Also, no colonies were formed in the presence of 50 mg/ml or 25 mg/ml of MTA, but lower concentrations did not show antifungal effect compared with the control (Figure 2).

After 48 h, the colonies were not seen in the presence of 50 mg/ml each of MTA and chlorhexidine, while 25 mg/ml of chlorhexidine or 50 mg/ml of calcium hydroxide had no antifungal effect. There was a direct correlation between the concentration of MTA and the inhibition of colony formation (K-W=20.66).

MTA and chlorhexidine (50 mg/ml each) effectively eliminated colony formation after three days (Table 1).

In contrast, lower concentrations of MTA and chlorhexidine did not inhibit colony formation, and the difference between the numbers of colonies on plates containing each 50 mg/ml MTA and chlorhexidine were significantly lower compared with plates with lower concentrations (p=0.0043). The data indicate that there was a significant difference in the antifungal effects of MTA, chlorhexidine, and calcium hydroxide at concentrations of 50 mg/ml (K-W=5.671, p=0.017) compared with 25 mg/ml (K-W=5.028, p=0.025) as a function of incubation time. MTA and 2% chlorhexidine in concentration of 50 mg/ml (MIC) were highly effective even after three days at 50 mg/ml (Figure 3).

Discussion

Because, yeasts mostly are present in the root canal together with bacteria [3,10], in this study was determined the minimal concentrations of calcium hydroxide, chlorhexidine, and MTA that inhibited the formation of colonies on agar plates by C. albicans. Because calcium hydroxide is used to treat radiologically confirmed periapical lesions, we chose to test it in the present study.

Current study found that calcium hydroxide began to lose its antifungal effect after 24 h and did not inhibit colony formation after 72 h. Ballal et al. [7] and Ferguson et al. [11] demonstrated that calcium hydroxide had no detectable antifungal effect. It has been demonstrated that C. albicans cells are highly resistant to Ca(OH)₂ and aqueous calcium hydroxide had no antifungal activity when maintained in direct contact with C. albicans cells [11].

These results may be explained by the insolubility of calcium hydroxide [7] and the low concentration used. Several studies show that C. albicans is resistant to calcium hydroxide but not to chlorhexidine [12-14]. It was reported that 2% chlorhexidine gel was significantly more effective than calcium hydroxide / 2% chlorhexidine mixture against C. albicans at 7 days, although there was no significant difference at 15 and 30 days. Calcium hydroxide alone was completely ineffective against C. albicans [15].

In this study, it was shown that 50 mg/ml of chlorhexidine effectively inhibited colony formation by C. albicans after 72 h. This may be explained by chlorhexidine's immediate bactericidal action as well as its prolonged bacteriostatic action due to its adsorption by enamel-coated surfaces and its ability to penetrate deep into dentin tubules [8,16].

We show here that the minimum inhibitory concentration (MIC) of MTA was 50 mg/ml. The mechanism of action of MTA involves the dissolution of calcium oxide, which increases pH by releasing Ca⁺² and OH⁻, which increase pH [17]. Moreover, our results are consistent with those of Al-Nazhan and Al-Judai [18] and Al-Hezaim et al. [19] who showed that the minimum inhibitory concentration of MTA against C. albicans after 72 h was 50 mg/ml [18,19]. Bidar et al. [20] found that MTA showed antifungal activity against Candida albicans and the

### Table 1: C. albicans growth after 72 hours.

| Concentration mg/ml | Groups | n (%) of cases with C. albicans colonies growth |
|---------------------|--------|---------------------------------------------|
|                     | Ca(OH)₂ | CHX 2% | MTA | Positive control | Negative control |
| 50      | 5 (100.0%) | 0 (0.0%) | 0 (0.0%) | - | 0 (0.0%) |
| 25      | 5 (100.0%) | 5 (100.0%) | 5 (100.0%) | - | 0 (0.0%) |
| 12.5    | 5 (100.0%) | 5 (100.0%) | 5 (100.0%) | - | 0 (0.0%) |
| 0.78    | 5 (100.0%) | 5 (100.0%) | 5 (100.0%) | - | 0 (0.0%) |
| Without tested material | - | - | 5 (100.0%) | - | - |
most antimicrobial activity of MTA was found to be against Candida albicans. Also, they reported about antibacterial increasing activity after addition of chlorhexidine to MTA. In contrast Estrela et al. found that the antifungal activity of MTA against C. albicans is limited to 48 h [21].

Because endodontic infections involve diverse microbes, drugs that are effective against a microorganism in vitro may not be effective in vivo. Therefore, studies in vivo are required to confirm the effects of calcium hydroxide, chlorhexidine, and MTA reported here.

Conclusions

We conclude that 72 h exposure to MTA and 2% chlorhexidine inhibits the formation of colonies in agar by C. albicans.

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