

In Vitro Antifungal Effect of Biodentine™ Against *Candida Albicans*

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Abstract

The aim of this in vitro study was to evaluate the antifungal efficacy and to determine the minimal inhibition concentration (MIC) of Biodentine™ against *Candida albicans*.

Sabouraud dextrose agar plates containing various concentrations of Biodentine™ powder were prepared. The plates were inoculated with an overnight culture of *C. albicans*, then observed for the formation of colonies after incubation at 37 °C for 1, 24, 48, or 72 h. The minimal concentration at which inhibition of the microorganism occurred was measured and noted as the MIC.

There was a statistically significant difference in the antifungal effect between the concentrations of 50 mg/ml, 25 mg/ml, and 12.5 mg/ml and the lower concentrations of Biodentine™ ($p < 0.05$). The MIC of Biodentine™ against *C. albicans* was 12.5 mg/ml.

We found that Biodentine™ in concentrations of 50 mg/ml, 25 mg/ml, and 12.5 mg/ml inhibited the growth of *C. albicans* in agar within three days.

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Introduction

Two of the most important factors for a successful endodontic treatment are the removal and elimination of microorganisms and the development of a fluid-tight seal.^{1,2} In cases where a primary root canal therapy fails and root canal retreatment cannot be performed, endodontic surgery procedures are applied.³ These surgeries are performed at the apex of the tooth, and they involve placement of the material at the apical end of the root to seal the root canal from the periapex. Most endodontic failures are due to inadequate cleaning of the root canal and a invasion of bacteria and other pathogens into the periradicular area. Along with bacteria, fungi are commonly present in the root canal.^{4,5} *Candida albicans* is one of the most commonly isolated fungal species in the oral cavity.⁶

Retrograde filling material should: 1) be biocompatible with the tissues that it contacts, 2) allow sufficient sealing, 3) induce bone

development or stimulate bone development, and 4) possess antimicrobial effects.⁷ The choice of material is important for a successful clinical outcome. To be fungicidal, biomaterials should show a strong alkaline reaction.^{8,9} The use of biomaterials in endodontic treatments has recently become more common. The dentin substitute Biodentine™ is a fast-setting calcium silicate-based restorative material that is suitable for direct restorative posterior filling, furcal perforation, retrograde filling, and pulp capping.¹⁰ Several in vivo and in vitro studies have reported its bioactivity and its successful performance in multiple clinical applications.¹¹⁻¹⁴

To evaluate the antimicrobial activity of materials, various laboratory methods can be used, including the agar dilution method. This method is an efficient means of evaluation because it involves a direct contact between the microorganisms and the experimental materials, which allows a more realistic interaction.¹⁵ Using this method, the minimal inhibition concentration (MIC), which is the lowest concentration of a material that prevents visible growth of microbes, can be evaluated.¹⁶

The aim of this in vitro study was to determine the MIC of Biodentine™ against *C. albicans*.

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Materials and methods

Five plates of Sabouraud dextrose agar (Liofilchem, Rosetodegli Abruzzi, Italy) were prepared for each tested concentration of Biodentine™ powder (Septodont, Saint Maur des Fosse's, France), which ranged from 50 mg/ml to 0.78 mg/ml. The agar mixed with the highest concentration was serially diluted with liquified molten agar (45 °C), and each time the Biodentine™ concentration was halved. Each concentration of the Biodentine™ agar compound was thoroughly mixed, and the uniform mix was poured into sterile Petri dishes and allowed to set completely.

Three plates of SDA without Biodentine™ served as positive control groups, and three plates without *C. albicans* served as negative control groups. The inoculum (10 µl per plate of *C. albicans* (ATCC10231, Liofilchem, Roseto degli Abruzzi, Italy) was prepared by growing an overnight culture from a stock culture (Figure 1), then using this culture to form a suspension with a turbidity equivalent to 0.5 McFarland opacity standards. After the plates were inoculated, they were placed in an incubator (Innovens 53, Jouan, France) set to 37 °C. The resulting fungal colonies on each plate were observed after 1, 24, 48, and 72 h (Figures 2 and 3).

The results were evaluated and analyzed with a Kruskal-Wallis test at a 95% level of confidence.

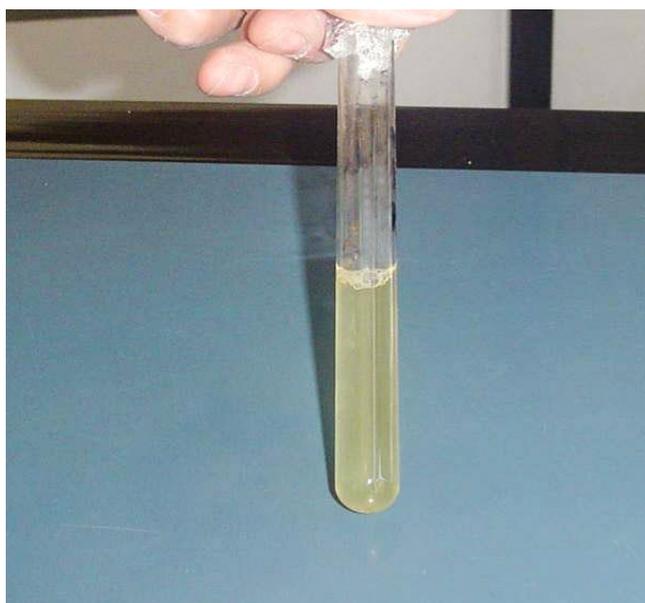


Figure 1. *Candida albicans* inoculum by growing an overnight culture from a stock culture.



Figure 2. No colonies of *Candida albicans* in 12.5 mg/ml Biodentine concentration after 72 h.



Figure 3. Colonies growth in 1.56 mg/ml Biodentine concentration.

Results

At each time period (1 h, 24 h, 48 h, and 72 h after inoculation) the presence of *C. albicans* colonies was assessed and recorded. The antifungal activities of the different Biodentine™ concentrations, as compared via a Kruskal Wallis, are shown in Table 1.

N=5 Concentration mg/ml	N (%) of cases with <i>C. albicans</i> colonies growth			
	1h	24h	48h	72h
50	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
25	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
12.5	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
6.25	0(0.0%)	0(0.0%)	5(100%)	5(100%)
3.12	0(0.0%)	5(100%)	5(100%)	5(100%)
1.56	0(0.0%)	5(100%)	5(100%)	5(100%)
0.78	0(0.0%)	5(100%)	5(100%)	5(100%)

Table 1. *Candida albicans* growth according to Biodentine concentrations and period of observations.

A direct correlation was found between the Biodentine™ concentration and its inhibitory effect on *C. albicans* growth. Plates containing Biodentine™ in a concentration of 50 mg/ml, 25 mg/ml, or 12.5 mg/ml showed significantly better antifungal activity, in all tested periods, compared with those containing lower Biodentine™ concentrations (KW=6.81, p=0.00902). Plates with the three highest tested Biodentine™ concentrations did not show *C. albicans* growth colonies at 72 h. Plates containing 6.25 mg/ml Biodentine™ showed antifungal activity only up through 24 h, but this concentration lost its antifungal activity by 48 h. There was a statistically significant difference within same concentration between 24 h and 48 h (KW=14.28, p=0.00254). Plates containing Biodentine™ concentrations of <6.25 mg/ml did not show any antifungal activity. Based on these results, the MIC of Biodentine™ against *C. albicans* appears to be 12.5 mg/ml for the period up to 72 h.

Discussion

C. albicans was selected for this study because it is often found in mixed cultures together with bacteria and is a common microorganism that often survives mechanical preparation, chemical procedures, and root canal medicaments.^{17,18} Fungal strain that was used in current study represents strain that is extensively used for clinical and laboratory research.¹⁹ Siqueira and Sen reported that *C. albicans* can colonize root canal walls and can easily penetrate dentin tubules.²⁰

Biodentine™, an endodontic repair material, possesses several advantageous properties that include good sealing capability, biocompatibility, and antibacterial activity.²¹ Here, we tested the antifungal activity of Biodentine™ via the agar dilution method and determined the MIC of Biodentine™ against *C. albicans*. To do so, the growth of *C. albicans* in concentrations of Biodentine™ ranging from 50–0.78 mg/ml was assessed for periods of up to 3 days. A direct correlation was found between the Biodentine™ concentration and the level of antifungal activity. The results show that a 3-day exposure to 50 mg/ml, 25 mg/ml, or 12.5 mg/ml Biodentine™ was sufficient to eradicate *C. albicans*.

Previous work evaluating and comparing various endodontic materials found that Biodentine™ showed a relatively strong antifungal

effect. For example, Bhavana et al. examined the antifungal activity of Biodentine™ via agar diffusion tests, and their results show that Biodentine™ exhibited a higher antifungal effect against *C. albicans* compared with mineral trioxide aggregate and glass ionomer cement.²²

Additionally, Demiryürek et al. found that all tested retrograde filling materials, including MTA Angelus, Biodentine™, and DiaRootBioAggregate, exhibited antibacterial and antifungal effects.²³ Furthermore, other work concluded that, regarding their antifungal effects, all evaluated biomaterials, including ProRoot MTA, MTA Plus, and Biodentine™, were suitable for use in endodontic treatment.²⁴ Biodentine alone seemed to have a greater inhibitory effect than the Biodentine/ chlorhexidine mixture on *C. albicans*, although the magnitude of the difference was very small.²⁵

Although many studies have investigated the antifungal effect of Biodentine™, there is only one report of the MIC of Biodentine™ against *C. albicans*. Hiremath et al. similarly reported that Biodentine™ exhibited a strong antimicrobial activity against *C. albicans*.²⁶ However, they found that the MIC of Biodentine™ was 2.5 mg/ml. This difference from our result may be due the differences in methodology between the two studies because they used the broth dilution method in which 200 mg of Biodentine™ was dissolved in 20 ml of sterile broth. The antifungal effect of Biodentine™ observed in both our study and previous work may be due to its high pH, given that high alkalinity is known to have an inhibitory effect on the growth of colonies and to disinfect dentin.

In contrast with our findings, Jardine et al., who investigated the antimicrobial effect of bioceramic cements on biofilm, reported that none of their tested materials, including Biodentine™, were effective on multispecies microcosm biofilms.²⁷ This apparent conflict may be explained by the differences in the diffusion capacity, contact properties, molecular weight, size, type, and concentration of Biodentine™ or differences in the properties of the agar medium.²⁸

Notably, in vitro studies of dental materials cannot always appropriately imitate real in vivo conditions, because of saliva, pH changes, temperature changes, food, liquids, and masticatory functions in the oral cavity.^{29,30} Thus, further in vitro and in vivo studies are required to

confirm the apparent antifungal activity of Biodentine™ against *C. albicans*.

Conclusions

Under the in vitro conditions of this study, Biodentine™ concentrations of 50, 25, and 12.5 mg/ml were effective in inhibiting the growth of *C. albicans* colonies for a period of up to three days. Lower concentrations of Biodentine™ were not effective.

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Declaration of Interest

The authors deny any conflict of interest related to this study.

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