# The Effect of Bovine Serum Albumin on the Antifungal Activity of Mineral Trioxide Aggregate Cements

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**Citation**

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**Abstract**

The aim of this study was to evaluate the effect of bovine serum albumin (BSA) on the antifungal effect of gray-colored MTA (GMTA) and white-colored MTA (WMTA) using a tube-dilution test. MTA preparations were tested freshly mixed and after 24 h on Candida albicans. The experiment was performed in 24-well culture plates. Fifty wells were used and divided into two experimental groups (freshly-mixed WMTA, freshly-mixed WMTA plus BSA, freshly-mixed GMTA, and freshly-mixed GMTA plus BSA) of 10 wells each and control groups of five wells each. Plates of Sabouraud dextrose agar mixed with C. albicans served as positive control and Sabouraud dextrose agar without C. albicans served as negative control. Fresh inoculate of C. albicans was prepared by growing an overnight culture from a stock culture. Aliquots of C. albicans were then taken from the stock culture and plated on the agar compound of the experimental and positive control group. All plates were incubated at 37°C for1h, 24 h, and 72 h. Growth of fungi was monitored daily by the presence of turbidity. Results showed the inhibitory effect of BSA on the antifungal effect of MTA cements during 24-h and 72-h incubation, whereas, there was no significant difference between 1-h incubation groups. It was concluded that the antifungal effect of freshly mixed MTA cements was decreased in the presence of BSA.

## INTRODUCTION

Microorganisms play an essential role in pulpal and periapical disease ([1,2,3]). Throughout the past decades, it has been well known that yeasts can be isolated from infected root canals. The occurrence of yeasts reported in infected root canals varies between 1% and 17 %([4]).

It has been shown that fungi colonization resulting in radicular pathosis can be associated with failing root canal treatments ([5,6,7,8,9]). The most commonly recovered fungi were Candida albicans ([5, 9, 10]). C. albicans also showed the ability to colonize root canal walls and penetrate into dentinal tubules.

Factors affecting the colonization of the root canal by fungi are not fully understood. However, it seems that among the predisposing factors of this process are certain intracanal medicaments, local and systemic antibiotics, and previous unsuccessful endodontic treatment. It has been hypothesized that the reduction of specific bacteria in the root canal during endodontic treatment may allow fungi overgrowth in the low nutrition environment. Further, fungi, such as C. albicans may gain access to the root canal because of coronal leakage ([4]).

Failures of initial endodontic treatment can often be successfully treated by orthograde retreatment or endodontic surgery. Elimination of the microbial flora and infected tissue as well as complete seal of the root canal system, to prevent future recontamination, will enhance treatment success ([11]). Mineral trioxide aggregate (MTA) has become a popular material to seal off communications between the root canal system and external surface of the root. It has been mostly used as a retrograde filling material and as a sealant of root perforations ([12]).

MTA (Pro Root MTA, Dentsply/Tulsa Dental, Tulsa, OK) is marketed in gray – coloured and white – coloured preparations; both are 75% Portland cement, 20% bismuth oxide, and 5% gypsum by weight ([11]). In recent years, the use of the white – coloured preparation became more popular. MTA is a powder that consists of fine hydrophilic particles that in the presence of water or moisture forms a colloidal gel that solidifies to form hard cement within approximately 3h ([12]). The main components of the gray – coloured formula are tricalcium oxide, tricalcium silicate, bismuth oxide, dicalcium, silicate, tricalcium aluminate, tetracalcium aluminoferrite, and calcium sulfate dihydrate. The white-colored preparation, however, lacks tetracalcium

aluminoferrite ([11]). A number of studies have revealed the inhibitory effect dentin, dentin matrix, bovine serum albumin (BSA), and hydroxyapatite on the antimicrobial activity of several intracanal medicaments (i.e. calcium hydroxide). Considering the fact that calcium hydroxide is the main chemical compound produced by MTA cements in aqueous environments, evaluation of inhibitory effect of bovine serum albumin (BSA) on the antimicrobial effects of these cements seems to be interesting. The purpose of this study was to assess the inhibitory effect of BSA on the antifungal effects of MTA cements against C. albicans in vitro.

## MATERIALS AND METHODS

The antifungal activity of white – coloured MTA (Pro Root MTA, Dentsply/Tulsa Dental, Tulsa, OK) as well as gray – coloured MTA (Pro Root MTA, Dentsply/Tulsa Dental, Tulsa, OK) was evaluated against Candida albicans. Stock cultures of clinically isolated C. albicans provided by the Microbiology Laboratory of Sadoughi University (Yazd, Iran) 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 mm screw – capped test tube and then incubated at 37°C for 1 week. Bovine serum albumin 18% (w/v) (BSA; Sigma® Chemical Co., St. Louis, MO, USA) was used as an organic load. The experiment was performed in plastic tissue – culture clusters containing 24 wells each with an inner diameter of 16 mm. A total of fifty wells were used and divided into four experimental groups (freshly-mixed WMTA, freshly-mixed GMTA, freshly-mixed WMTA plus BSA, and freshly-mixed GMTA plus BSA) of 10 wells each and control groups of five wells each. In the experimental group 1, 1 g of WMTA was mixed at the bottom of each culture well. In the experimental group 2, 1 g of GMTA was mixed at the bottom of each culture well. In groups 3 and 4, freshly mixed WMTA plus BSA, and freshly mixed GMTA plus BSA were used respectively. One milliliter sterile BSA was added to the wells in groups 3 and 4 and was thoroughly mixed with a sterile pipette before adding the fungal inuculum. For the positive control group, 1 ml of Sabouraud infusion – broth media was mixed with 1 ml of Candida suspension in a culture well. In the negative control group, 2 ml of Sabouraud broth infusion was placed in culture well. The culture plates of all experimental and control groups were then incubated anaerobically at 37°C and evaluated at 1, 24 and 72 h time periods. At each time 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 incubated at 37°C and monitored for the consecutive 7 days. Growth of the fungi was monitored daily by the presence of turbidity in the tubes. The results were analyzed statistically using Kruskal – Wallis test.

## RESULTS

The negative control showed no fungal growth in all experimental periods, whereas the positive control demonstrated entirely fungal growth, which confirms the method.

Evaluation of the freshly mixed MTA groups without BSA demonstrated fungal growth during the 1-h incubation of C. albicans with WMTA as well as GMTA. However, by increasing the incubation time, there was no growth in 24 h and 72 h. In the freshly mixed MTA groups containing BSA fungal growth was observed during all incubation periods. Statistically, there was no significant difference between GMTA and WMTA ($P>0.05$). However, the difference between groups containing BSA and groups without BSA was statistically significant ($P<0.05$). All cell culture wells had the same results in each experimental group.

## DISCUSSION

The method used in the present 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 ([13]). This method allows direct contact between fungal cells and the MTA material. Sabouraud agar is a commonly used medium for the isolation of oral yeasts. The pH of the medium is quite acidic (usually 5.6) allowing the growth of yeasts and aciduric organisms, whereas most bacteria are inhibited ([4]).

C. albicans frequently associated with failing root canal treatments, has the ability to form biofilm on different surfaces ([5]). This property is one of the reasons why this species is considered to be more pathogenic than species that are less able to form biofilm ([5]).

It is generally known that organic material can inactivate or weaken the effect of calcium hydroxide. Portenier et al. ([14]) showed that killing of Enterococcus faecalis by calcium hydroxide was completely prevented by 18% (w/v) BSA. Lower concentrations of BSA were not tested, and it is therefore unclear whether BSA is an equally effective inhibitor of the antimicrobial activity of calcium hydroxide as dentin powder. Considering the fact that MTA has a

soluble fraction mainly composed of calcium hydroxide, it is interesting to evaluate the inhibitory effect of BSA on the antifungal activity of MTA cements.

The results of the present study showed that freshly mixed and 24 –h set MTA cements without BSA were effective against C. albicans in 24 and 72 – h time periods. Al – Nazhan and Al – Judai ([15]) showed that white – coloured MTA to be effective in killing C. albicans in vitro for a period of up to 72h. On the other hand, Estrella et al. ([16]) found that the antifungal activity of gray – coloured MTA against C. albicans was limited during a 48 – h period.

In another in vitro study, Sipert et al. ([17]) found that antifungal effect of MTA was not significantly different from Portland cement, but was significantly lesser from root canal sealers. To date, there is no evidence of the antifungal activity of MTA against C. albicans for periods longer than 3 days. However, it has been shown that MTA has a soluble fraction mainly composed of calcium hydroxide and the water in contact with MTA had a high alkaline PH ranging of 11.94 to 11.99([18]). A long – term study demonstrated that MTA did maintain a high pH for 78 days ([19]). It is therefore possible that MTA and calcium hydroxide possess similar antimicrobial action. In this regards, several studies evaluated the susceptibility of C. albicans to calcium hydroxide. Waltimo et al. ([20]) studied the susceptibility of common oral Candida species to saturated aqueous calcium hydroxide solution. They found that the sensitivity of the C. albicans strains was relatively low for short – term exposure, however, after 6 –h incubation, 99.9% of Candida strains were killed. On the other hand, Barbosa et al. ([21]) found that a saturated solution of calcium hydroxide was effective in killing C. albicans already after 3 min of incubation. Siqueira et al. ([22]) evaluated the antifungal activity of different intracanal medicaments against C. albicans and found that calcium hydroxide completely eliminated the fungus from bovine Dentin after 7 days. They also found that a combination of calcium hydroxide and paramonochlorophenol eliminated C. albicans within 1h. Estrella et al. ([16]) reported that an aqueous preparation of calcium hydroxide paste in saline was more effective in inhibiting C. albicans growth than MTA or Portland cement. Al – Hezaimi et al. ([11]) evaluated the antifungal activity of different concentrations of white – coloured MTA on C. albicans in vitro in various exposure periods. Their findings showed a direct correlation between MTA concentration and its inhibition effect on the growth of C. albicans .MTA in concentration of 50mg/ml inhibited the growth of C. albicans in any of the time periods tested (1, 24, 48, 72h). In concentration of 25mg/ml, MTA inhibited the growth of C. albicans at 1 and 24 – h time periods. In lower concentrations, MTA did not inhibit the growth of C. albicans.

Extrapolation of the results of this in vitro study to clinical situations must be done with caution. Sealing ability and biocompatibility of MTA are more important from the clinical point of view. Further, the 72 h evaluation of the MTA cements is not sufficient for any conclusions to be drawn about their antifungal effects. Therefore, it is suggested that the antifungal activity of MTA cements be investigated for longer periods of time.

In conclusion, within the limits of the present study, antifungal activity of white – colored as well as gray – colored MTA was completely inhibited in the presence of BSA.

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