



# The Open Dentistry Journal

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## RESEARCH ARTICLE

### Antifungal Effectiveness between Tricalcium Silicate-White Portland Cements Added $\text{Bi}_2\text{O}_3$ and Mineral Trioxide Aggregate Against *Candida albicans*

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#### Abstract:

##### Background

*Candida albicans* is the most dominant fungus found in root canal reinfection cases. This microorganism can withstand extreme pH, low oxygen levels, lack of nutrients, and penetrate the dentinal tubules so that it can resist the intracanal medicament. Root canal cement helps prevent microorganisms and causing root canal reinfection. MTA is one of the root canals cement which is widely used and has an excellent antifungal activity, but it is less beneficial economically. Tricalcium silicate-white Portland cement (WPC) has a similar main composition as MTA, except there is no  $\text{Bi}_2\text{O}_3$  content that functions as a radiopacifier.

##### Objectives:

To fabricate a mixture of tricalcium silicate-WPC with  $\text{Bi}_2\text{O}_3$  through a simple solution method and investigate antifungal activity's effectiveness between tricalcium silicate-WPC added  $\text{Bi}_2\text{O}_3$  and MTA to *C. albicans*.

##### Materials and Methods:

The 80 g of tricalcium silicate-WPC was mixed with 20 g of  $\text{Bi}_2\text{O}_3$  through the simple solution method using 99.9% isopropanol as a solution. This sample solution is stirred until homogeneous, then centrifuged. The precipitate was dried until a dry powder was obtained. The powder was analyzed using X-Ray fluorescence spectrometry to identify its chemical composition and concentration. A total of 30 samples were divided into two experimental groups of tricalcium silicate-WPC added  $\text{Bi}_2\text{O}_3$  and ProRoot MTA. The vials, which contain cement and *C. albicans*, respectively, were incubated at 37°C for 24 hours and diluted to obtain a suspension  $10^4$ ,  $10^6$  (0.5 in McFarland's nephelometer) and then inoculated with sterile cotton swabs onto Saboroud Dextrose Agar Plates. The plates were incubated at 37°C for 24 hours. The measurement of colony number of *C. albicans* was counted by colony counter (CFU/ml).

##### Results:

The  $\text{Bi}_2\text{O}_3$  was revealed in tricalcium silicate-WPC based on XRF characterization, and the antifungal test showed that both materials were effective against *C. albicans*. There was no statistically significant difference in the number of *C. albicans* colonies between tricalcium-WPC added  $\text{Bi}_2\text{O}_3$  and MTA ( $p > 0.05$ ).

##### Conclusion:

The mixture of  $\text{Bi}_2\text{O}_3$  in tricalcium silicate-WPC was successfully fabricated through a simple solution method, and both samples were effective against the *C. albicans*.

**Keywords:** Tricalcium silicate-white portland cement,  $\text{Bi}_2\text{O}_3$ , MTA, *Candida albicans*, Chemical composition, Colony counter (CFU/ml).

#### Article History

Received: August 18, 2020

Revised: December 02, 2020

Accepted: December 16, 2020

## 1. INTRODUCTION

The failure factor in endodontic treatment is caused by

persistent microorganisms in the root canal [1 - 7]. Fungi are microorganisms that can be found in secondary infections with

an incidence of between 2% and 40% other than bacteria. The results showed that *Candida albicans* is the most dominant type of fungi found in endodontic re-treatment [6, 7]. *C. albicans* is a fungus that can be able to withstand extreme pH, low oxygen levels, and lack of food sources that make microorganisms resistant in the root canal [8]. These microorganisms penetrate the dentinal tubules and interact with other microorganisms to form biofilm complexes and cause reinfection in the root canals [9].

Sealer is an essential material in the stage of filling the root canal as a filling material between guttapercha and the root canal walls and filling gaps and irregularities in the root canal [10, 11]. The ideal cement must-have antimicrobial activity to suppress the remaining microorganisms in the root canal [12 - 14]. Mahmoud Torabinejad first developed Mineral Trioxide Aggregate (MTA) in 1993 with the main components of tricalcium silicate, tricalcium aluminate, tetra calcium aluminophosphate, and  $\text{Bi}_2\text{O}_3$ . The high alkalinity of MTA can inhibit the growth of microorganisms. Several studies have shown the antibacterial effect of MTA on *Enterococcus faecalis* and *C. albicans* [15 - 20]. MTA is still not widely used because the price is relatively high. Previous research showed the similarity of biocompatibility, sealing ability, and dimensional stability [21 - 23]. Tricalcium silicate Portland cement produced by Indocement, Ltd., Cirebon, West Java, consists of grey and White Portland Cement (WPC). Tricalcium silicate-WPC has the same composition as MTA, which contains calcium aluminate and calcium sulfate with lower iron content without  $\text{Bi}_2\text{O}_3$  [24, 25]. Bismuth oxide ( $\text{Bi}_2\text{O}_3$ ) is a radiopaque material added to a root canal filling material to be seen radiographically. Based on the study results, Guerreiro *et al.* showed that the addition of  $\text{Bi}_2\text{O}_3$  to Portland cement did not change the pH and the antimicrobial effect [26 - 29].

This study aims to fabricate a mixture of tricalcium silicate-WPC with  $\text{Bi}_2\text{O}_3$  through a simple solution method and assess the difference in antifungal activity effectiveness between tricalcium silicate-WPC added  $\text{Bi}_2\text{O}_3$  and MTA against *C. albicans*.

## 2. MATERIALS AND METHODS

### 2.1. Synthesis of Tricalcium Silicate-WPC Added $\text{Bi}_2\text{O}_3$

At this stage, the mixing of tricalcium silicate-WPC (Indocement Ltd, Cirebon, Indonesia) was carried out with  $\text{Bi}_2\text{O}_3$  as followed: tricalcium silicate-WPC 80 g mixed with  $\text{Bi}_2\text{O}_3$  20 g was dissolved using 99.9% isopropanol as a solution. This sample solution is stirred using a magnetic stirrer for 30 minutes until homogeneous, then centrifuged. The precipitate was dried using a vacuum drying oven for 60 minutes until a dry powder was obtained.

### 2.2. Characterization X-Ray Fluorescence (XRF)

The mixture of tricalcium silicate -WPC added  $\text{Bi}_2\text{O}_3$  was

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analyzed using X-Ray Fluorescence spectrometry (ED-XRF Rigaku Nex CG, The Woodlands, USA) to identify their chemical composition and concentration.

### 2.3. Antifungal Test

In this study, tricalcium silicate-WPC (Indocement Ltd, Cirebon, Indonesia) added  $\text{Bi}_2\text{O}_3$  and ProRoot MTA (Dentsply Sirona, Charlotte, USA) were employed. *C. albicans* -ATCC 10231 provided by Microbiology Laboratory of Pharmacy School, ITB, Bandung. A total of 30 samples were divided into two experimental groups of tricalcium silicate-WPC added  $\text{Bi}_2\text{O}_3$  and ProRoot MTA.

Tricalcium silicate-WPC added  $\text{Bi}_2\text{O}_3$  (0.5 g) inserted into a vial of *C. albicans* suspension (5 ml) and gently shaken to ensure direct contact between fungi and cement as well as ProRoot MTA. The vials were incubated at 37°C for 24 hours and diluted to obtain a suspension  $10^4$ ,  $10^6$  (0.5 in McFarland's nephelometer) and then inoculated with sterile cotton swabs onto Saboroud Dextrose Agar Plates. The plates were incubated at 37°C for 24 hours. The measurement of colony number of *C. albicans* was counted by colony counter (CFU/ml).

## 3. RESULTS

### 3.1. Composition of Tricalcium Silicate-WPC Added $\text{Bi}_2\text{O}_3$

The chemical compositions of the tricalcium silicate-WPC added  $\text{Bi}_2\text{O}_3$  by XRF are shown in Table 1. The main chemical compounds of tricalcium silicate-WPC added  $\text{Bi}_2\text{O}_3$  were Calcium (Ca) 56.7%, Silica (Si) 14.6%, Bismuth (Bi) 10.6%, Magnesium (Mg) 10.1%, and Aluminum (Al) 5.2% as seen in (Fig. 1). Other minerals were detected under 1% called trace elements. Compared with ProRoot MTA, the amount of Calcium was relatively higher than ProRoot MTA, as well as Aluminum and Magnesium. Tricalcium silicate-WPC added  $\text{Bi}_2\text{O}_3$  contained a small amount of silica (Si) than ProRoot MTA. Metal elements were found in both materials, tricalcium silicate-WPC added  $\text{Bi}_2\text{O}_3$  and ProRoot MTA at different concentrations. Tricalcium silicate-WPC added  $\text{Bi}_2\text{O}_3$  contained a lower amount of iron (Fe) than ProRoot MTA. Zirconium (Zr) was found only in tricalcium silicate-WPC added  $\text{Bi}_2\text{O}_3$  at a low concentration.

### 3.2. Antifungal Activity

Antifungal activity was assessed with the measurement of colony number *C. albicans* (CFU/ml) used colony counter, as present in Table 2 and the mean number of CFU/ml for each material against *C. albicans* is shown in Table 3. The growth of *C. albicans* colonies before and after applying tricalcium silicate-WPC added  $\text{Bi}_2\text{O}_3$  and ProRoot MTA incubated for 24 hours is shown in (Figs. 2-4). Among the tested materials, tricalcium silicate-WPC added  $\text{Bi}_2\text{O}_3$  and ProRoot MTA present a similar result. There was no statistically significant difference between the fungi count of the Tricalcium silicate-WPC added  $\text{Bi}_2\text{O}_3$  and ProRoot MTA after 24 hours incubated since  $p > 0.05$ , as shown in Table 4.

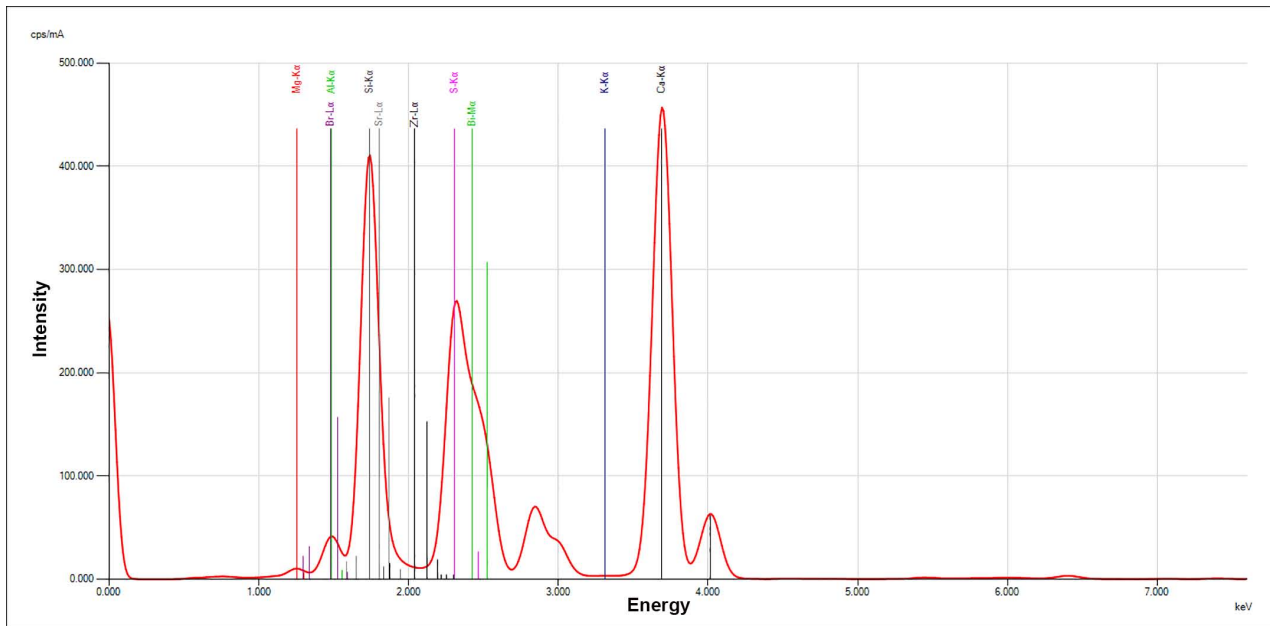


Fig. (1). XRF peak of tricalcium silicate-WPC added Bi<sub>2</sub>O<sub>3</sub> sample.

Table 1. Chemical compositions of Tricalcium silicate-WPC added Bi<sub>2</sub>O<sub>3</sub> and ProRoot MTA based on X-Ray Fluorescence Spectrometer (XRF).

Minerals	White Portland Cement-Bi <sub>2</sub> O <sub>3</sub> (mass%)	ProRoot MTA[26, 27](mass%)
Iron (Fe)	0.2	0.38
Magnesium (Mg)	10.1	5.16
Potassium (K)	0.3	0.45
Calcium (Ca)	56.7	54.6
Titanium (Ti)	0.05	0.09
Chrom (Cr)	0.003	0.003
Aluminum (Al)	5.2	1.19
Zirconium (Zr)	0.7	-
Sulphur (S)	1.0	1.96
Silica (Si)	14.6	17.6
Bismuth (Bi)	10.6	14.46
Strontium (Sr)	0.3	0.22

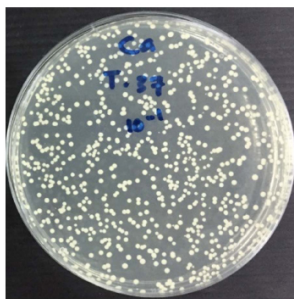


Fig. (2). The growth of *Candida albicans* colonies before the application of Tricalcium silicate-WPC added Bi<sub>2</sub>O<sub>3</sub> and ProRoot MTA.

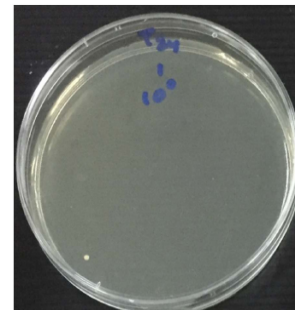


Fig. (3). The growth of *Candida albicans* colonies after application of Tricalcium silicate-WPC added Bi<sub>2</sub>O<sub>3</sub> incubated for 24 hours.

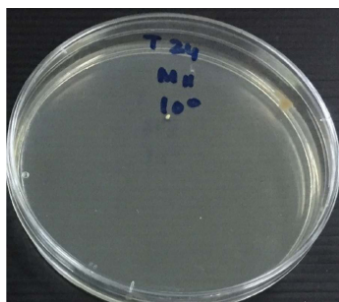


Fig. (4). The growth of *Candida albicans* colonies after the application of ProRoot MTA incubated for 24 hours.

Table 2. The *Candida albicans* colonies number after application with Tricalcium silicate-WPC added Bi<sub>2</sub>O<sub>3</sub> and ProRoot MTA for 24 hours.

Tricalcium silicate-WPC added Bi <sub>2</sub> O <sub>3</sub> (CFU/ml)	ProRoot MTA (CFU/ml)
1	2
1	0
0	1
2	1
2	0
0	1
0	1
0	0
1	0
1	1
0	1
1	0
0	1
0	0
1	0

Table 3. Means of antifungal effectiveness of Tricalcium silicate-WPC added Bi<sub>2</sub>O<sub>3</sub> and ProRoot MTA for 24 hours.

-	Means number of colonies <i>Candida albicans</i> (CFU/ml)
Tricalcium silicate-WPC added Bi <sub>2</sub> O <sub>3</sub>	0.6
ProRoot MTA	0.6

Table 4. An Independent t-test between a group of Tricalcium silicate-WPC added Bi<sub>2</sub>O<sub>3</sub> and ProRoot MTA before and after 24 hours.

-	Before 24 h	After 24 h	
		Tricalcium silicate-WPC added Bi <sub>2</sub> O <sub>3</sub>	ProRoot MTA
Averages	88 x 10 <sup>5</sup>	0.667	0.600
Standard deviation	990.430	0.724	0.632
n	15	15	15
p-value		0.067*	

\*p-value > 0.05.

#### 4. DISCUSSION

Microorganisms are the main factors causing endodontic treatment failure [1 - 7]. *C. albicans* are the most dominant fungi found in the root canals and can cause endodontic treatment failures [8, 9]. *C. albicans* have properties that survive in the root canal after biomechanical preparation and root canal medication [10, 14]. Therefore, the obturation is an essential stage so that a root canal cement with antifungal activity and good sealing ability is needed to prevent microorganisms from entering and causing infection after root canal treatment is completed [15, 16]. MTA is one of the root canals cement widely used and has some ideal properties as a sealer, one of which is an excellent antifungal activity [17 - 20].

MTA's main content is tricalcium silicate, dicalcium silicate, tricalcium aluminate, and tetra calcium aluminoferrite, also found in Portland cement [20 - 25]. The similarity of the main content has been studied to provide similar properties in both materials, including the ability of its antifungal activity [26 - 29]. Tricalcium silicate-WPC also has the same content as MTA [30, 31]. The difference between the two materials lies in the content of Bi<sub>2</sub>O<sub>3</sub> [16, 18, 20]. Bi<sub>2</sub>O<sub>3</sub> is a radiopacifier that can increase radiopacity so that it can be seen radiographically [20, 21]. In this study, Bi<sub>2</sub>O<sub>3</sub> was added to Tricalcium silicate-WPC by 20% according to the amount of Bi<sub>2</sub>O<sub>3</sub> in the MTA. According to Marina *et al.* (2014) and Paulo (2012), the addition of Bi<sub>2</sub>O<sub>3</sub> to Portland cement has been investigated as a function of a radiopacifier and does not affect pH and the antimicrobial activity which are owned by Portland cement [22, 24]. Guerreiro (2012) also adds the radiopacifiers in Portland cement, which do not change their properties [25].

XRF characterization analysis on tricalcium silicate-WPC added Bi<sub>2</sub>O<sub>3</sub> showed some differences in mineral composition and concentration. The amount of calcium present in tricalcium silicate-WPC added Bi<sub>2</sub>O<sub>3</sub> is higher than MTA, thus maximizing the release of hydroxyl ions obtained when hydration reaction and affect *C. albicans*. The metal elements are also found in both samples, such as iron (Fe), Magnesium (Mg), Titanium (Ti), Chrom (Cr) and Strontium (Sr) with different concentrations. Tricalcium silicate-WPC added Bi<sub>2</sub>O<sub>3</sub> has a lower iron (Fe) than MTA, minimizing the possibility of staining and toxicity. Iron is one of the heavy metal elements and has an intense color [25, 31, 32]. Tricalcium silicate-WPC added Bi<sub>2</sub>O<sub>3</sub> has magnesium and aluminum higher compared to MTA. Magnesium (Mg) above 6% causes expansion of the material and reduces the risk of shrinkage and a gap formation after setting. Aluminum (Al) affects the setting time of the material. The aluminum found in the Tricalcium silicate-WPC added Bi<sub>2</sub>O<sub>3</sub> is higher than the MTA; this showed that the Tricalcium silicate-WPC added Bi<sub>2</sub>O<sub>3</sub> has a longer setting time than MTA. MTA has less aluminum and more iron (Fe). A decrease in setting time can be achieved by adding iron (Fe) and calcium chloride (CaCl) [14, 19, 36]. Zirconium (Zr) is found only in the Tricalcium silicate-WPC added Bi<sub>2</sub>O<sub>3</sub> and functions as a radioopacifier [32 - 36].

Dicalcium silicate and Tricalcium silicate contained in Tricalcium silicate-WPC when reacted with water (H<sub>2</sub>O) then

form calcium silicate hydrate ( $\text{CaO} \cdot 2\text{SiO}_2 \cdot 3\text{H}_2\text{O}$ ) and calcium hydroxide ( $\text{Ca}(\text{OH})_2$ ). Calcium hydroxide will break down into calcium ions ( $\text{Ca}^{2+}$ ) and hydroxyl ions ( $\text{OH}^-$ ). Hydroxyl ( $\text{OH}^-$ ) are active free radicals that can increase the pH level to 12.9 and affect inhibiting the growth of microorganisms [30, 31]. Jafari *et al.*, stated that MTA's antifungal activity comes from Alkaline pH and inhibit the growth of *C. albicans* [12]. Bhavana *et al.*, also stated that MTA contains calcium oxide, and when in contact with water, it will form calcium hydroxide and increase the pH up from 10.2 to 12.5 within 3 hours [37].

The ability of hydroxyl ions ( $\text{OH}^-$ ) inhibits microorganisms' growth through three stages, damaging the cytoplasmic membrane by inhibiting the synthesis of ergosterol found in fungi cell membranes, breaking the DNA chain, and inhibits the process of replication, and causes protein denaturation [38]. Hydroxyl ions are free radicals that can diffuse into the dentinal tubules and cause lethal effects on microorganisms [22, 23, 30, 32, 33]. *C. albicans* can live in the root canal with an optimal pH of 4.5-8. Environmental pH conditions that rise to alkaline can affect the cell's pH so that pH changes disrupted the activity of ergosterol synthesis enzymes [39, 40]. Hydroxyl ions cause protein denaturation in *C. albicans* cells. Optimal enzyme activity occurs at controlled pH. Alkalinity induces disrupted ionic bonds, which maintains the protein structure's stability and causes loss of biological activity in enzymes and disruption of cell metabolism [39, 40]. Hydroxyl ions also affected the DNA chain of *C. albicans* cells. Disrupted DNA chain causes inhibition of the replication of *C. albicans* cells so that the microorganism can not reproduce itself [39, 40].

## CONCLUSION

The mixture of  $\text{Bi}_2\text{O}_3$  with tricalcium silicate-WPC could be done through a simple solution method, and there was no statistically significant difference ( $p > 0.05$ ) in the effectiveness of the antifungal activity. In other words, the tricalcium silicate-WPC added  $\text{Bi}_2\text{O}_3$ , and ProRoot MTA had similar efficacy against the *C. albicans*.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are base on this research.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF DATA AND MATERIALS

The data sets analyzed during the current study are available from the corresponding author [A.C] on request.

## FUNDING

This research supported and funded by Universitas Padjadjaran with the grant number of 1427/UN6.3.1/LT/2020.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

## ACKNOWLEDGEMENTS

The authors would like to express special appreciation and thanks to Finders Centre of Excellence, Universitas Padjadjaran, and Integrated Laboratory of Faculty of Dentistry, Universitas Padjadjaran, Bandung, Indonesia.

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