

## ANTIMICROBIAL ACTIVITY OF MINERAL TRIOXIDE AGGREGATE AND CALCIUM HYDROXIDE SEALER ON *ENTEROCOCCUS FAECALIS* STRAIN ATCC29212

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### ABSTRACT

**Objective:** The objective of this study was to evaluate the antimicrobial activity of mineral trioxide aggregate (MTA) and calcium hydroxide (Ca(OH)<sub>2</sub>) sealers on *Enterococcus faecalis* at various time points after the preparation of the sealer.

**Methods:** Antimicrobial activity of MTA and Ca(OH)<sub>2</sub> sealer on *E. faecalis* (ATCC 29212) was assessed by the direct contact test immediately, 1 day, and 7 days after the preparation of the sealer.

**Results:** The antimicrobial effect of MTA sealer was greatest 1 and 7 days after the preparation of the sealer. The effect of Ca(OH)<sub>2</sub> was greatest 1 day after the preparation of the sealer.

**Conclusion:** MTA sealer showed constant antimicrobial activity on *E. faecalis* overtime. By contrast, Ca(OH)<sub>2</sub> sealer showed decreasing antimicrobial activity overtime, with the greatest antimicrobial activity being 1 day after the preparation of the sealer.

**Keywords:** Antimicrobial activity, Calcium hydroxide sealer, *Enterococcus faecalis*, Mineral trioxide aggregate sealer.

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### INTRODUCTION

*Enterococcus faecalis* is a facultative anaerobic microbe present in 23–70% of cases of failed endodontic treatment with periapical lesion [1]. Successful endodontic treatment is achieved by the endodontic triad: Access preparation, cleaning and shaping, and obturation. Since all the microbes in root canals cannot be eliminated by chemomechanical preparation, hermetic obturation with an antimicrobial filler and sealer material is needed to eliminate the remaining microbes [2,3].

Calcium hydroxide (Ca(OH)<sub>2</sub>) sealer, which is biocompatible, has limited antimicrobial activity [4,5]. The mineral trioxide aggregate (MTA) sealer has good adhesion, hydrophilic, biocompatibility, and antimicrobial properties [6]. The MTA sealer contains calcium oxide and releases Ca(OH)<sub>2</sub> when it contacts fluid. Ca(OH)<sub>2</sub> breaks down into hydroxyl ions and increases the pH level. This process is repeated over a long time period, making the MTA sealer more advantageous than Ca(OH)<sub>2</sub> sealer. In a previous study, *E. faecalis* was resistant at pH level of 9.5–10, inhibited at pH 10.5–11, and died at ≥11.5 [7–10].

This study aimed to analyze the antimicrobial activity of MTA and Ca(OH)<sub>2</sub> sealers on *E. faecalis* immediately, 1 day, and 7 days after the preparation of the sealer. The hypothesis of this study is that the MTA sealer has better antimicrobial activity than the Ca(OH)<sub>2</sub> sealer due to the properties described.

### METHODS

This experimental study was performed at the Oral Biology Laboratory in the Faculty of Dentistry, University of Indonesia. The materials used included *E. faecalis* strain ATCC29212, AnaeroGen Compact 5, MTA sealer Fillapex, Ca(OH)<sub>2</sub> Apexit Plus sealer, agar blood, brain heart infusion (BHI) broth, Bacto agar, NaCl 0.9%, and 0.5 McFarland standard. The instruments used were beaker glass, Eppendorf tubes, Epis, incubator, colony counter unit, object glass, ballpoint, tangle, and Petri dish.

### *E. faecalis* microbial culture

Microbial suspension was cultured on brain heart agar plates with BHI broth and was incubated under anaerobic conditions at 37°C for 2 × 24 h. The microbial colony turbidity was adjusted using a 0.5 McFarland standard solution (1 × 10<sup>8</sup> colony-forming unit [CFU]/ml).

### Sample preparation

Ca(OH)<sub>2</sub> sealer (Apexit Plus) and MTA sealer (MTA Fillapex) were placed homogeneously on object glass under humid conditions at 37°C and treated immediately, 1 day, and 7 days after the preparation of the sealer.

### Direct contact test

The sealer was placed on the side of the Epis and, then, 0.01 cc microbial suspension was placed on top immediately, 1 day, or 7 days after the preparation of the sealer. Then, the samples were incubated for 1 h with 100% humidity at 37°C. 240 µL BHI broth was added to each sample, was homogenized, and was diluted 10-fold. For each sealer, a 10 µL solution was inoculated on an agar plate and was incubated at 37°C for 24 h. Following the incubation, the CFUs were counted. The number of CFUs for each sealer for each time period was analyzed by a parametric one-way analysis of variance (ANOVA) and *post hoc* tests. The statistical significance was set at p<0.05.

### RESULTS

Table 1 presents the number of CFUs that formed after direct contact between *E. faecalis* and the sealer immediately, 1 day, and 7 days after the preparation of the sealer. For both the MTA and the Ca(OH)<sub>2</sub>, the lowest number was observed 1 day after the preparation of the sealer.

One-way ANOVA revealed significant differences between antimicrobial activity of the MTA and Ca(OH)<sub>2</sub> across the three time points (p<0.05). *Post hoc* tests (Table 2) indicated significant differences between *E. faecalis* colony numbers for the MTA immediately, 1 day, and 7 days after the preparation of the sealer. The MTA sealer placement 1 day and 7 days after preparation decreased *E. faecalis* number more than

**Table 1: Average and standard deviation of CFUs formed after direct contact between *E. faecalis* and the MTA or the Ca (OH)<sub>2</sub> sealer immediately, 1 day, and 7 days after the preparation of the sealer**

Test group	n	Mean±SD	95% confidence interval	
			Minimum	Maximum
MTA				
Immediately	16	575.40±187.10	475.74	675.14
1 day	16	230.81±166.78	141.94	319.68
7 days	16	320.44±226.75	199.61	441.26
Ca (OH) <sub>2</sub>				
Immediately	16	766.31±255.46	630.19	902.44
1 day	16	405.38±343.33	222.43	588.32
7 days	16	619.81±383.91	415.24	824.38

*E. faecalis*: *Enterococcus faecalis*, MTA: Mineral trioxide aggregate, CFUs: Colony-forming units, Ca (OH)<sub>2</sub>: calcium hydroxide

**Table 2: Significance values of *E. faecalis* colony numbers between MTA sealer and Ca (OH)<sub>2</sub> sealer immediately, 1 day, and 7 days after the preparation of the sealer**

Test group I	Test group II	p value
MTA immediately	MTA 1 day	0.001*
	MTA 7 days	0.010*
	Ca (OH) <sub>2</sub> immediate	0.050
MTA 1 day	MTA 7 days	0.354
	Ca (OH) <sub>2</sub> 1 day	0.073
MTA 7 days	Ca (OH) <sub>2</sub> 7 days	0.003*
Ca (OH) <sub>2</sub> immediately	Ca (OH) <sub>2</sub> 1 day	0.000*
	Ca (OH) <sub>2</sub> 7 days	0.132
Ca (OH) <sub>2</sub> 1 day	Ca (OH) <sub>2</sub> 7 days	0.028*

\*Post hoc analysis, \*significance value p<0.05. *E. faecalis*: *Enterococcus faecalis*, MTA: Mineral trioxide aggregate, Ca (OH)<sub>2</sub>: calcium hydroxide

immediate placement. The best antimicrobial activity for the MTA occurred 1 day and 7 days after the preparation of the sealer, with no significant difference between the two time points. For Ca(OH)<sub>2</sub>, placement 1 day after preparation showed a significantly greater decrease than immediate placement or placement 7 days after preparation. 1 day after preparation, the ability of the MTA sealer to decrease *E. faecalis* colonies was equal to that of the Ca(OH)<sub>2</sub> sealer. 7 days after preparation, the MTA sealer had better antimicrobial activity than Ca(OH)<sub>2</sub>.

## DISCUSSION

*E. faecalis* is a pathogenic microbe found in 38% of failed endodontic treatment cases [11]. This microbe can resist extreme conditions and a lack of oxygen and nutrition and can form a biofilm matrix that inhibits antimicrobial agents [2,11-13].

The Ca(OH)<sub>2</sub> sealer is biocompatible and releases hydroxyl ions, creating a basic environment that eliminates microbes. However, the antimicrobial activity is limited because it cannot maintain a high pH for a long period [14]. The MTA sealer has high biocompatibility and works as a Ca(OH)<sub>2</sub> sealer. When it contacts tissue fluid, the MTA releases calcium ions that react with phosphate ions, creating calcium phosphate. Calcium phosphate forms carbonated apatite, which is a layer between the MTA and dentin, similar to hydroxyapatite. This layer hermetically seals the root canal, thereby eliminating remaining microbes. The MTA sealer is composed of calcium oxide which, every time it contacts water, creates Ca(OH)<sub>2</sub> that breaks into calcium and hydroxyl ions, thereby maintaining a high pH in the tissue [15-18].

The Ca(OH)<sub>2</sub> antimicrobial activity is achieved by disrupting microbial cytoplasmic membranes through lipid LTA peroxidation of *E. faecalis* cell membranes, resulting in protein denaturation and the inhibition of microbial DNA replication [19].

The antimicrobial activity of MTA sealer 1 day and 7 days after preparation was the best among the MTA sealer group. This is supported by Ustun *et al.* [20,21] who showed that MTA sealer was not effective on *E. faecalis* 20 min after the preparation of the sealer but was effective 1 day and 7 days after the preparation of the sealer. The Ca(OH)<sub>2</sub> releasing process in MTA occurs every time that there is contact with tissue fluid; therefore, the pH remained high causing a constant antimicrobial activity until 7 days after the preparation of the sealer [18,22-24]. Fridland showed that MTA can maintain a pH high of 11-12 for up to 78 days [25]. The low antimicrobial activity in MTA sealer immediately after preparation was affected by the initial setting time (2.27±0.06 h) and the final setting time (4.55±0.05 h). As soon as the sealer was prepared, the setting reaction that formed Ca(OH)<sub>2</sub> had not yet occurred [26]. This is supported by a previous study showing that the MTA and the Ca(OH)<sub>2</sub> sealer at 1 day had an equal ability to decrease *E. faecalis* colony. Rather, antimicrobial activity was affected by the Ca(OH)<sub>2</sub> formed after setting [27].

The number of *E. faecalis* colonies after contact with the Ca(OH)<sub>2</sub> sealer 1 day after preparation was lower than for the immediately after and 7 days after groups. This finding supports that the Ca(OH)<sub>2</sub> antimicrobial activity occurred after setting, which took 2 h 15 min, and continuously decreased overtime [28]. This pattern is supported by Zhang *et al.* [29] who showed that Ca(OH)<sub>2</sub> could not eliminate microbes in the first 60 min after the preparation of the sealer and had not yet completed the setting reaction. Slutzky-Goldberg [30] showed that Ca(OH)<sub>2</sub> sealer had a short-term antimicrobial effect of 1 day on *E. faecalis*. The Ca(OH)<sub>2</sub> antimicrobial activity depended on hydroxyl ions and was effective if the pH remained high. However, Ca(OH)<sub>2</sub> could not create hydroxyl ions once it had broken down completely. Hence, the pH decreased overtime, from 12.5 to 9.14. This caused a decrease in Ca(OH)<sub>2</sub> antimicrobial activity on *E. faecalis*, which can resist a pH >11.5 [11,19].

## CONCLUSION

The MTA sealer had better antimicrobial effects than Ca(OH)<sub>2</sub> sealer did by direct contact on *E. faecalis* immediately, 1 day, and 7 days after the preparation of the sealer.

## CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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