# The MIC and MBC of calcium hydroxide medicament against bacteria that cause a chronic periapical abscess in the vulnerable initial 7-days of endodontic treatment

**Chany Mony Dwiayu Putri[1], Diani Prisinda[1*], Yuti Malinda[2]**

**[1]Department of Conservative Dentistry, Faculty of Dentistry Universitas Padjadjaran, Indonesia**
**[2]Department of Oral Biology, Faculty of Dentistry Universitas Padjadjaran, Indonesia**

**ABSTRACT**

**Introduction:** The primary aetiology of chronic periapical abscesses, including E. faecalis, S. mutans, S. sanguinis, and P. gingivalis, can be eliminated using intracanal medicaments such as calcium hydroxide. The purpose of this study was to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) value of calcium hydroxide paste in the vulnerable initial 7-day of treatment against E. faecalis ATCC 29212, S. mutans ATCC 25175, S. sanguinis ATCC 10556, and P. gingivalis ATCC 33277. **Methods:** An in-vitro laboratory experiment using a spectrophotometer as a microdilution method was conducted to determine bacteria's MIC and MBC values on calcium hydroxide paste. The sample used in this study was four bacteria using intracanal medicament of calcium hydroxide (Ca(OH)2), incubated for seven days at 37°C, and then bacterial growth was observed. The cell inhibition percentage was calculated using optical density measurements to determine the MIC value. The low MIC and MBC were defined as sensitive bacteria to calcium hydroxide. **Results:** Calcium hydroxide paste against E. faecalis (ATCC 29212) with MIC values at a concentration of 750 µg/ml and MBC values at a concentration of 96,000 µg ml; S. mutans (ATCC 25175) with MIC value at a concentration of 3,000 ug/ml and MBC value at a concentration of 48,000 ug/ml; S. sanguinis (ATCC 10556) with MIC value at a concentration of 3,000 ug/ml and MBC value at a concentration of 6,000 ug/ml; P. gingivalis (ATCC 33277) with MIC value at a concentration of 6,000 ug/ml and MBC value at a concentration of 48,000 ug ml. **Conclusions:** Calcium hydroxide can inhibit bacterial growth activity. E. faecalis (ATCC 29212) and S. sanguinis (ATCC 10556) are more sensitive to calcium hydroxide paste than other bacteria, with the lowest MIC and MBC on seven days of incubation since the maximum calcium and hydroxyl ions are released.

**Keywords:** *E. faecalis; S. mutans; S. sanguinis; P. gingivalis;* calcium hydroxide; MIC; MBC

*p-ISSN: 1979-0201; e-ISSN: 2549-6212; Available from: http://jurnal.unpad.ac.id/pjd/article/view/28638*
*DOI: 10.24198/pjd.vol34no1.28638*
*Submission: July 17, 2020; Accepted: Mar 31, 2022; Published online: Mar 31, 2022*

*[*]Corresponding author: Diani Prisinda, Department of Conservative Dentistry, Faculty of Dentistry Universitas Padjadjaran, Indonesia. Sekeloa Selatan I Street, Bandung, West Java, 40132, Indonesia. Phone: +62 818-207-891; e-mail: diani.prisinda@fkg.unpad.ac.id*

## INTRODUCTION

Many Indonesians tend to see a dentist when they already have minor or severe dental problems. A survey conducted by Rahardjo et al.[1] stated that pulp and periapical disease are the eighth most common diseases found in hospitals. One of the periapical diseases often found is a periapical abscess.[1]

A periapical abscess is an inflammatory disease that occurs in periapical tissue. This condition is caused by a necrotic tooth which becomes an accumulating place for bacteria. This inflammation starts from the reaction that occurs in the pulp and spreads to the periapical tissue through the apical foramen. Inflammation started because of the accumulation of bacteria in the pulp, causing tooth pain and even necrosis. The necrotic tooth will trigger immune cells to kill bacteria so that it forms pus formation characterised by the high number of PMN cells, necrotic tissue, and dead bacterial cells. A periapical abscess is divided into acute (symptomatic) and chronic (asymptomatic).[2,3] Chronic periapical abscess is a persistent inflammation that is asymptomatic or has no symptoms caused by polymicrobial infections with large numbers of bacteria.[2,3]

Based on culture and molecular studies conducted by Siqueira et al.,[5] periapical abscess microbiota is a mixed bacteria dominated by anaerobic bacteria. *Streptococcus spp.* is the highest prevalence bacteria isolated from dentoalveolar abscesses, including periapical abscesses. Among *Streptococcus spp.*, *S. sanguinis* (6.6%) and *S. mutans* (10%) were found as the causes of periapical abscesses. In addition, it was also found that *P. gingivalis* (59%) and *E. faecalis* (5%) in root canals with periapical abscesses. Gram-positive bacteria in *cocci*, such as *S. mutans*, *S. sanguinis*, and *E. faecalis*, grow in facultative anaerobic conditions, where they use oxygen for growth but can independently use the adenosine triphosphate (ATP) fermentation process when oxygen in the tissue is low. Other bacteria that cause chronic periapical abscesses are *P. gingivalis* bacteria, obligate anaerobic gram-negative bacteria in the form of immobile *Coccobacilli*, which cannot survive without oxygen and are often becoming the main cause of disease in the oral cavity. [4,5,6,8,9]

The treatment procedure for pulp and periapical diseases based on the American Association of Endodontists is endodontic treatment with two visits, where intracanal medicament will be given at the first visit on the treatment that functions as antimicrobials for limiting bacterial growth and as a disinfectant and in the second visit the intracanal medicament will be observed to see if the intracanal medicament works, then the procedure will be continued with the obturation on the pulp.[2,7]

Intracanal medicament often used in endodontic treatment is calcium hydroxide ($Ca(OH)_2$). Calcium hydroxide is an odourless powder, paste, or gel, which is used as an intracanal medication in non-surgical conventional endodontic procedures. Calcium hydroxide has a high pH to increase the activity of alkaline phosphatase, which is one of the tooth elements for the mineralisation process that can effectively kill microbes by releasing hydroxyl ions to denature bacterial DNA. Calcium hydroxide, often used in dentistry, is in paste because of its easy use and good adaptation to tissue. One of the calcium hydroxide materials often used is UltraCal XS (Ultradent™) in a 1.2 ml syringe with good radiopacity and pH 12.5. Its composition consists of 41% water, 35% calcium hydroxide, 19% barium sulfate, 3% propylene glycol, and 2% methylcellulose. Calcium hydroxide paste (UltraCal XS) has an outstanding release of hydroxyl ions and calcium ions due to its high pH. Calcium hydroxide releases the hydroxyl and calcium ions as an antimicrobial, where the ions denature bacterial DNA, which causes the bacteria to die. The full release of ions occurs for seven days.[2,7,21]

The effectiveness of intracanal medicament as an antibacterial agent can be determined by the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). MIC defines as the lowest concentration of agents that inhibit the growth of bacteria, while MBC defines as the lowest concentration of an agent that can kill bacteria or bactericidal agents. MIC and MBC can be measured by measuring the turbidity or optical density (OD) at the specific wavelength using a spectrophotometer when mixing the bacteria and calcium hydroxide as an agent to observe the density of bacteria in solution.[8,9,22,23]

On the seventh day, the hydroxyl ions will begin to diffuse into the dentinal tubules and cause changes in the permeability of the dentin. This diffusion causes an increase in pH that induces the neutralisation of lactic acid in the tooth in the dentin tubules, thereby changing the integrity of the bacterial cytoplasmic membrane through the toxic effect produced in nutrient transfer.[24] Research conducted by Grover et al.[8] stated that calcium hydroxide is effective as an antimicrobial for 7 to 15 days to eliminate and/or reduce the number of bacteria remaining after biomechanical preparation. However, Sabrah et al.[9] declared that "The MIC and MBC values for Ca(OH)2 were 1:10 (1.6 mg/mL) for E. faecalis and 1:80 (0.2 mg/mL) for P. gingivalis. However, no MIC and MBC values for Ca(OH)2 were obtained in this study, which suggests a poor antimicrobial activity of the medicament"; therefore, the author considers calcium hydroxide is not very good at inhibiting bacteria based on its MIC and MBC values. The controversy and the purpose of this study are to analyse the effectivity of calcium hydroxide medicament against bacteria that cause a chronic periapical abscess in the vulnerable initial seven days of endodontic treatment by calculating the bacteria's MIC and MBC values.

## METHODS

The current study was an experimental laboratory using the microdilution method by determining the value of minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). This type of study was chosen because both sample and control were controlled, measured, and the influence of the control can be trusted.[15]

The sample used in this study was four samples for each bacteria using intracanal medicament of calcium hydroxide ($Ca(OH)_2$) with various concentrations, namely 192.000, 96.000, 48.000, 24.000, 12.000, 6.000, 3.000, 1.500, 750, 375, 187, 94 µg/mL with twice repetitions based on the Federer formula and incubated for seven days in 37°C. The controlled variables used in this study were sterile aquadest as solvents of calcium hydroxide. The research procedure stages were medium preparation, bacterial preparation, sample preparation and antibacterial testing. Therefore, the optical density measurement of the media, solvents, drug samples, and bacteria is measured using a spectrophotometer with a wavelength of 600nm. The formula's optical density values are used to get the MIC value using cell inhibition percentage.

The percentage of cell inhibition was obtained from the reduction between the optical density of solvent and bacterial media. Then divide by the optical density of the bacterial media, and multiply by one hundred percent.

The MIC value can be measured by determining the percentage of bacterial inhibition calculated by the optical density formula shown from the spectrophotometer. In contrast, the MBC procedure was calculated by taking a bacterial preparation from a percentage showing on the MIC value result, then the bacteria preparation was taken using oese, and then the bacteria was scratched on the MHA agar plate. The agar plate was incubated for 24 hours and observing the bacterial development in the agar plate. MBC values are claimed in which concentration there is no bacterial growth seen in the agar plate measured using a colonymeter. The lower MIC and MBC are defined as sensitive bacteria to calcium hydroxide.

## RESULTS

The effectiveness of calcium hydroxide pastes UltraCal XS against *E. faecalis* ATCC 29212, *S. mutans* ATCC 25175, *S. sanguinis* ATCC 10556, and *P. gingivalis* ATCC 33277 was measured using a spectrophotometer in order to observe the value of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The controlled substance used was sterile aquadest as a solvent for calcium hydroxide paste.

### *Antibacterial calcium hydroxide test against E. faecalis*

To determine the MIC value of calcium hydroxide against *E. faecalis* (ATCC 29212), the antibacterial test was conducted using a microdilution method with repeated dilution, starting at a 192,000 µg/ml concentration. The average absorbance value of each repeated dilution treatment was input to the cell inhibitory percentage formula—the data of turbidity values and percent cell inhibition values of calcium hydroxide against *E. faecalis*.

The percentage value of bacterial inhibition of calcium hydroxide is graphed to observe at which concentration has a significant increase in the percentage of cell inhibition to obtain the MIC value of calcium hydroxide paste against *E. faecalis*. For example, the percentage of *E. faecalis* inhibition against calcium hydroxide can be seen in Figure 1 at a concentration of 750 µg/ml, and a significant percentage begins to occur by evaluating the increase of cell inhibition value.

The antibacterial test was continued by observing the value of MBC through a visual observation on the bacterial culture, to determine at what concentration of calcium hydroxide was able to kill the *E. faecalis*. The MBC value was determined based on the visual observations, as presented in Figure 2, which appeared to look clean without any bacterial colonies growing at the 96,000 µg/ml and 192,000 µg/ml concentrations consecutively.

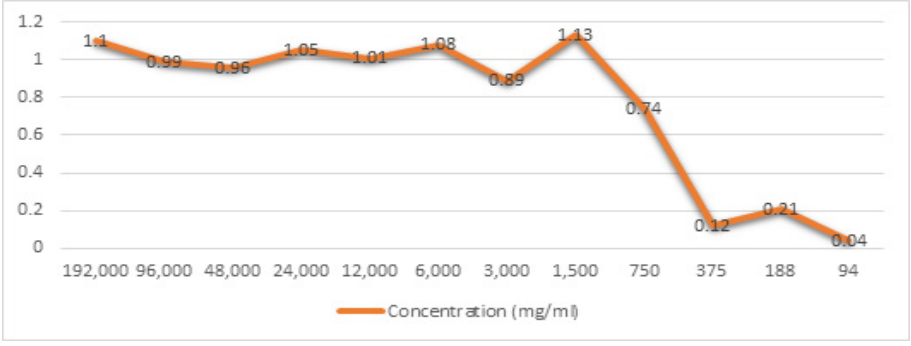


**Figure 1. The percentage value of *E. faecalis* inhibition of calcium hydroxide. The X-axis is the concentration of calcium hydroxide, and Y-axis is the percentage of *E. faecalis* cell inhibition**

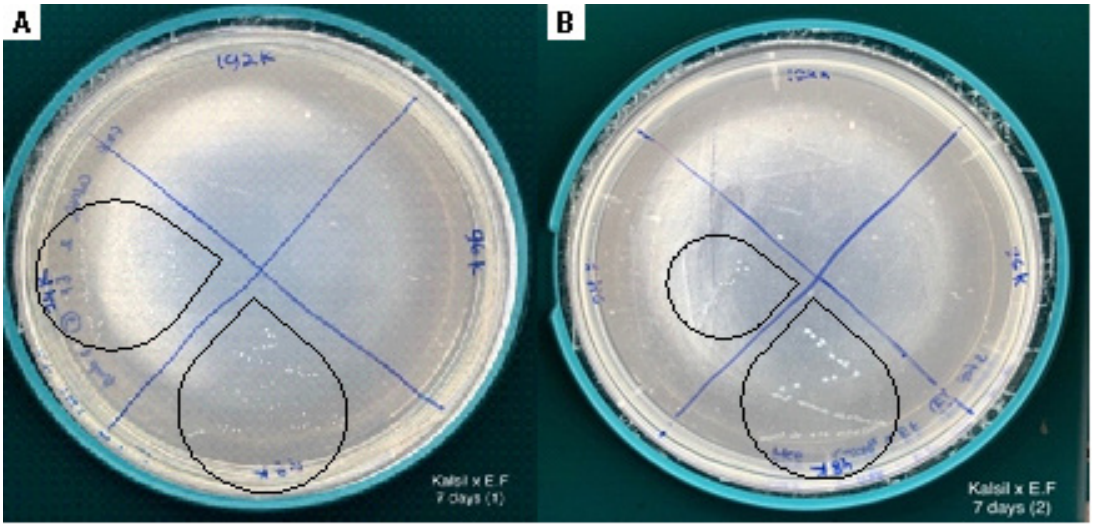


**Figure 2. Two replicated of the MBC values of calcium hydroxide against *E. faecalis*: a) The first medium looked clean without any bacterial colonies growing starting at the lowest concentrations of 96,000 µg/ml; b) The second medium also looked clean without any bacterial colonies growing at the same concentrations as the first medium**

***Antibacterial calcium hydroxide test against S. Mutans***

The MIC value against *S. mutans* (ATCC 25175), was determined with a microdilution method was performed with repeated dilution starting at a 192,000 µg/ml concentration. The average absorbance value of each treatment was input to the cell inhibitory percentage formula—the data on turbidity values and percent inhibitory value of cells from calcium hydroxide against *S. mutans*.

The percentage value of bacterial inhibition of calcium hydroxide is graphed to determine at which concentration has a significant increase in the percentage of cell inhibition to obtain the MIC value of calcium hydroxide paste against *S. mutans*. For example, the percentage of *S. mutans* inhibition against calcium hydroxide can be seen in Figure 3 at a concentration of 3,000 µg/ml, and a significant percentage begins to occur by evaluating the increase of cell inhibition.

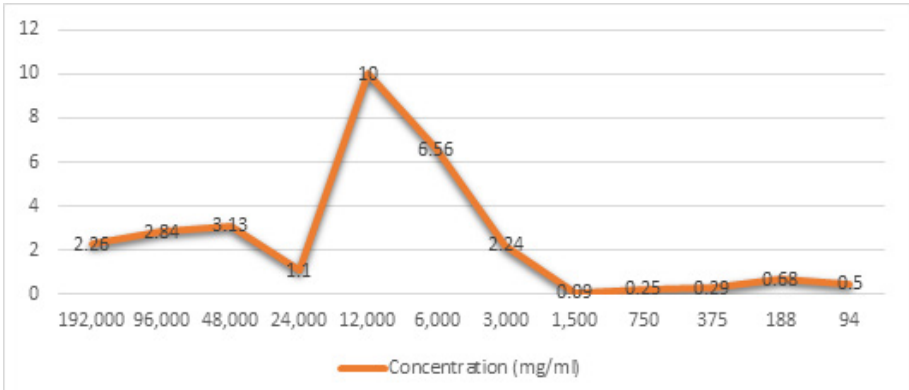


**Figure 3. The percentage value of *S. mutans* inhibition of calcium hydroxide. The X-axis is the concentration of calcium hydroxide, and Y-axis is the percentage of *S. mutans* cell inhibition**

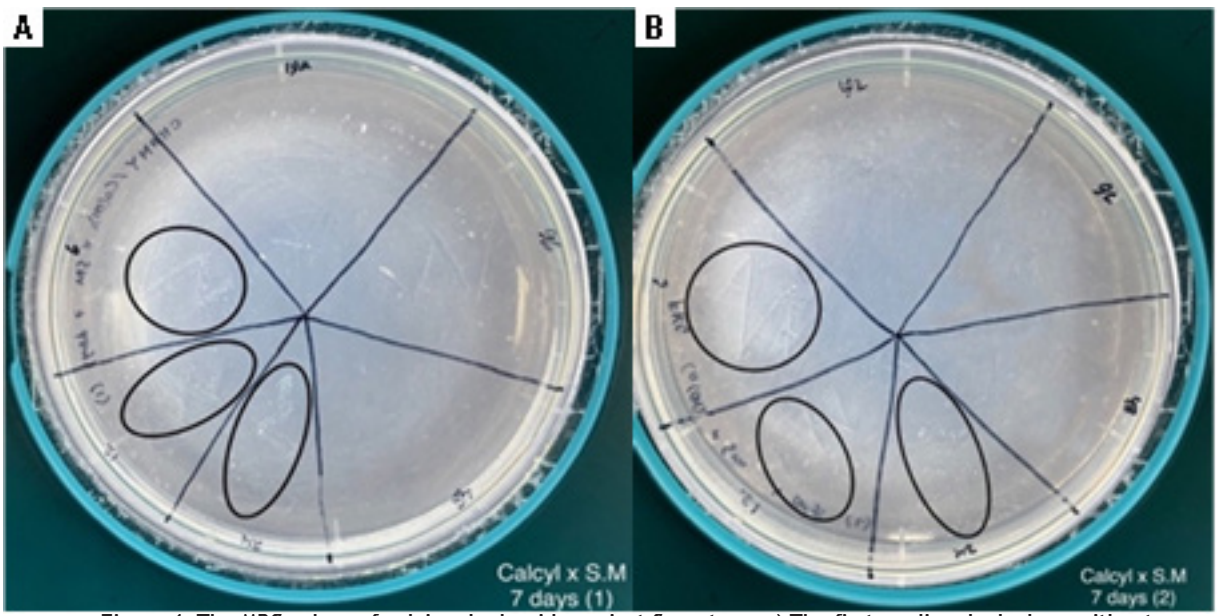


**Figure 4. The MBC values of calcium hydroxide against *S. mutans*: a) The first medium look clean without any bacterial colonies growing start at the lowest concentrations of 48,000 µg/ml; b) The second medium also lok clean without any bacterial colonies growing at the same concentrations as the first medium**

The antibacterial test was continued by observing the value of MBC by visuals in culture to find out at what concentration of calcium hydroxide can kill *S. mutans* bacteria. The MBC values based on visual observations in Figure 4 appear clean without any bacterial colonies growing at concentrations of 48.000 µg/ml, 96.000 µg/ml, and 192.000 µg/ml.

***Antibacterial calcium hydroxide test against S. sanguinis***

To determine the MIC value of calcium hydroxide against *S. sanguinis* (ATCC 10556), the antibacterial test used a microdilution method with repeated dilution starting at a concentration of 192.000 µg/ml. The average absorbance value of each treatment was input to the cell inhibitory percentage formula—the data on turbidity values and percent inhibitory value of cells from calcium hydroxide against *S. sanguinis*.

The percentage value of bacterial inhibition of calcium hydroxide is graphed to observe at which concentration has a significant increase in the percentage of cell inhibition to obtain the MIC value of calcium hydroxide paste against *S. sanguinis*. For example, the percentage of *S. sanguinis* inhibition against calcium hydroxide can be seen in Figure 5 at a concentration of 3,000 µg/ml, and a significant percentage begins to occur by evaluating the increase of cell inhibition.

The antibacterial test was continued by observing the value of MBC by visuals in culture to find out at what concentration of calcium hydroxide can kill *S. sanguinis* bacteria. The MBC values based

on visual observations in Figure 6 appeared clean without any bacterial colonies growing starting at concentrations of 6.000 µg/ml, 24.000 µg/ml, 48.000 µg/ml, 96.000 µg/ml, and 192.000 µg/ml.

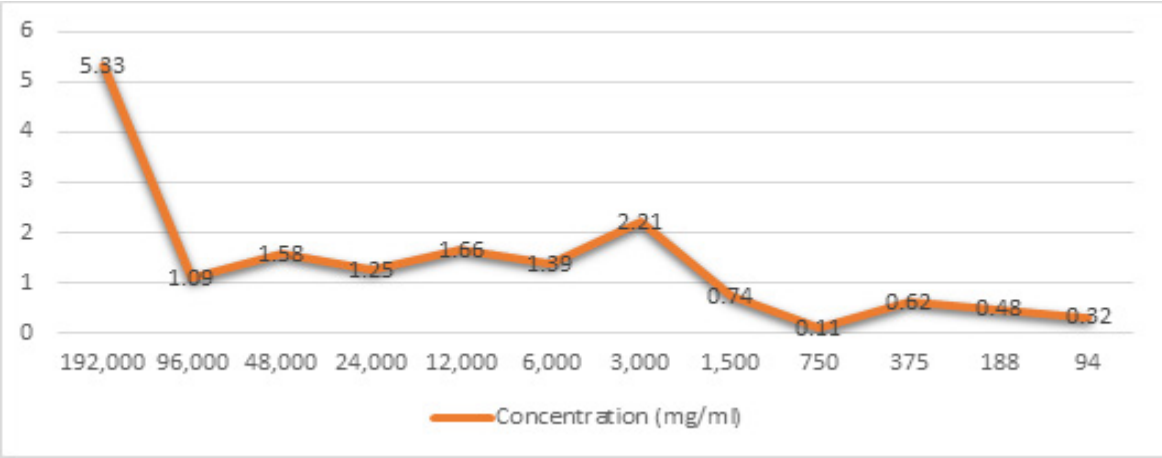


**Figure 5. The percentage value *S. sanguinis* inhibition of calcium hydroxide. The X-axis is the concentration of calcium hiydroxide, Y-axis is the percentage of *S. sanguinis* cell inhibition**

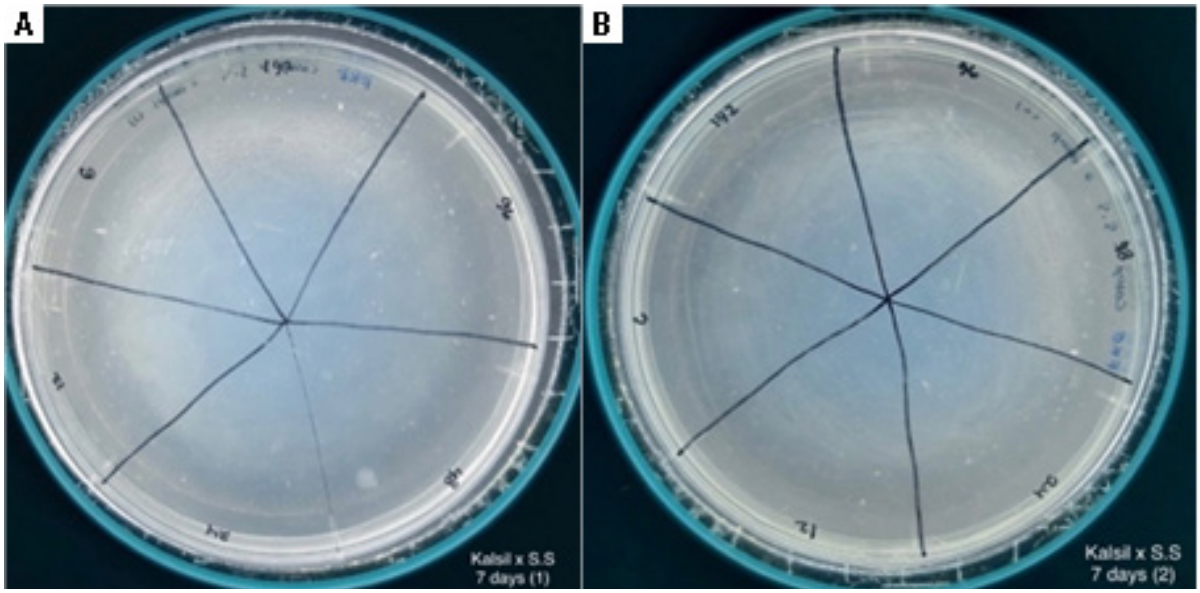


**Figure 6. The MBC values of calcium hydroxide against *S. sanguinis*: (a-b) Both of the medium look clean without any bacterial colonies growing start at the lowest concentrations of 6000 µg/ml.**

### *Antibacterial calcium hydroxide test against P. gingivalis*

To determine the MIC value of calcium hydroxide against *P. gingivalis* (ATCC 33277), the antibacterial test used a microdilution method with repeated dilution starting at a concentration of 192.000 µg/ml. The average absorbance value of each treatment was input to the cell inhibitory percentage formula—the data on turbidity values and percent inhibitory value of cells from calcium hydroxide against *P. gingivalis*.

The percentage value of bacterial inhibition of calcium hydroxide is graphed to observe at which concentration has a significant increase in the percentage of cell inhibition to obtain the MIC value of calcium hydroxide paste against *P. gingivalis*. For example, the percentage of *P. gingivalis* inhibition against calcium hydroxide can be seen in Figure 7 at a concentration of 6,000 µg/ml, where a significant percentage begins to occur by evaluating the increase of cell inhibition.

The antibacterial test was continued by observing the value of MBC by visuals in culture to find out at what concentration of calcium hydroxide can kill *P. gingivalis*. The MBC values based on visual observations in Figure 8 appeared clean without any bacterial colonies growing at concentrations of 48.000 µg/ml, 96.000 µg/ml, and 192.000 µg/ml.

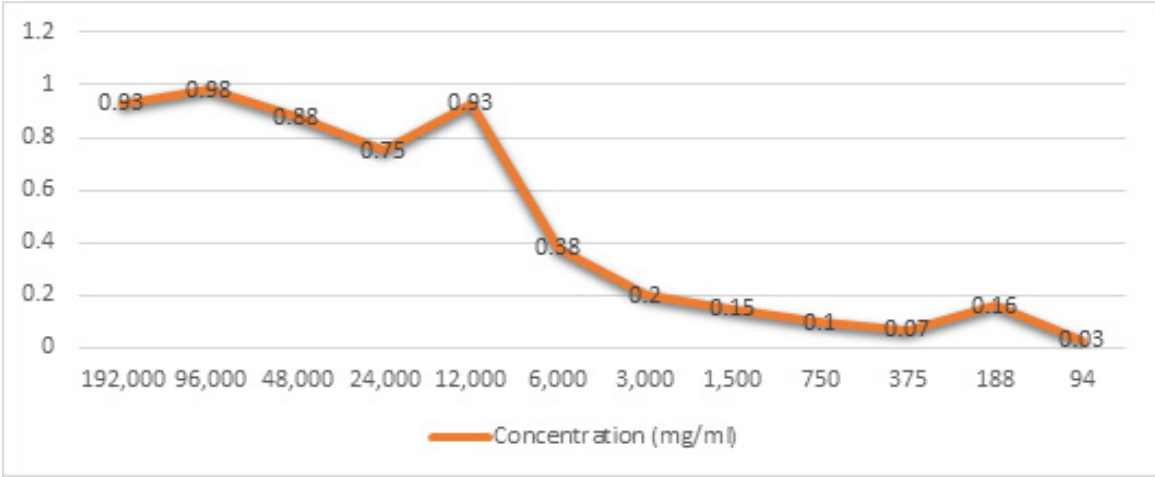


**Figure 7. The percentage value of *P. gingivalis* inhibition of calcium hydroxide. The X-axis is the concentration of calcium hiydroxide, and Y-axis is the percentage *P. gingivalis* of cell inhibition**

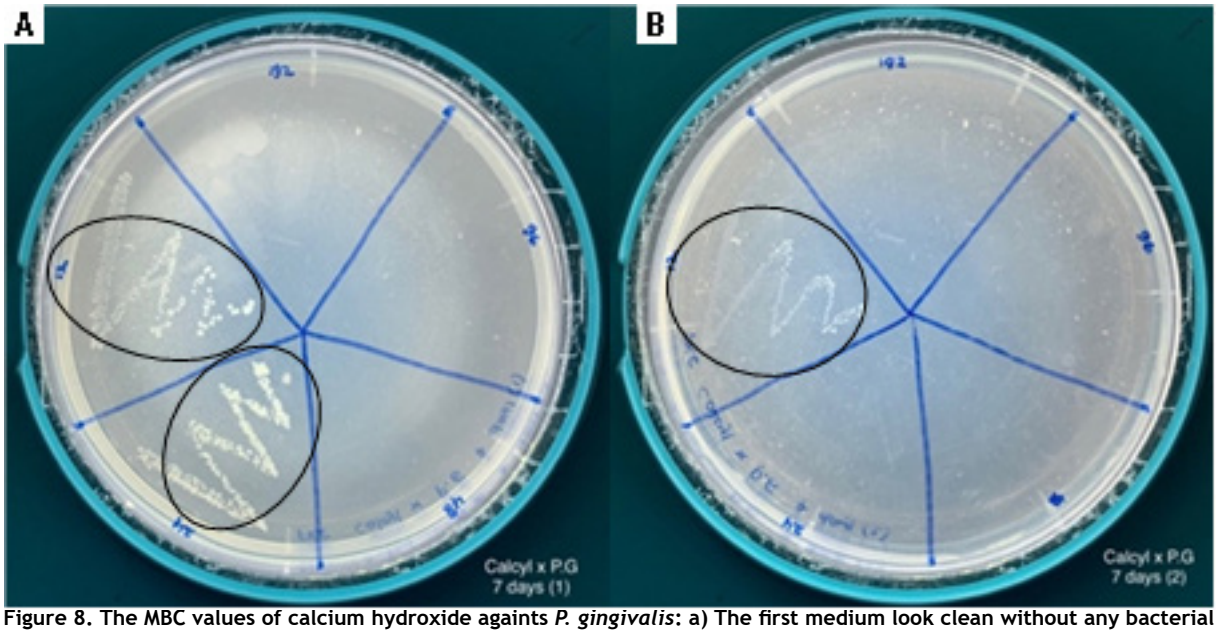


**Figure 8. The MBC values of calcium hydroxide againts *P. gingivalis*: a) The first medium look clean without any bacterial colonies growing start at concentrations of 48,000 µg/ml; b) The second medium also look clean without any bacterial colonies growing start at concentrations 24,000 µg/ml.**

**Table 1. The MIC and MBC values from calcium hydroxide paste**

| Bacteria | Paste preparation of calcium hydroxide | |
|---|---|---|
| | MIC (µg/ml) | MBC (µg/ml) |
| *E. faecalis* | 750 | 96,000 |
| *S. mutans* | 3,000 | 48,000 |
| *S. sanguinis* | 3,000 | 6,000 |
| *P. gingivalis* | 6,000 | 48,000 |

## DISCUSSION

The percentage graph of inhibition of bacterial growth based on the MIC values in Figures 1, 3, 5, and 7, also based on the MBC values in Figures 2, 4, 6, and 8, show that calcium hydroxide could inhibit bacterial growth activity and kill bacteria following the research conducted by Grover et al.[8] that calcium hydroxide is effective as an antibacterial in 7 days. This experimental study also obtained that calcium hydroxide can inhibit bacterial growth activity and kill bacteria seen from the MIC value. The MBC visual result which *E. faecalis* (ATCC 29212) and S. mutans (ATCC 25175)

has the lowest MIC value at a concentration of 750 µg/ml, and *S. sanguinis* (ATCC 10556) has the lowest MBC value at a concentration of 6.000 µg/ml. Sabrah et al.[9] said that "The MIC and MBC values for $Ca(OH)_2$ were 1:10 (1.6 mg/mL) for *E. faecalis* and 1:80 (0.2 mg/mL) for *P. gingivalis*. However, no MIC and MBC values for $Ca(OH)_2$ were obtained in this study, which suggests a poor antimicrobial activity". Besides the results given priorly, we saw that for *E. faecalis*, the MIC and the MBC values were 0.75 mg/mL and 96 mg/mL.

Gram-positive bacteria such as *E. faecalis*, *S. mutans*, *S. sanguinis*, and gram-negative bacteria such as *P. gingivalis* can cause virulence factors by forming biofilms, which produce lipoteichoic acid as a toxic enzyme which form an attachment to the bacteria that caused resistance effects to disinfectants, antibiotics, and immune responses.[15] Calcium hydroxide can inhibit biofilms by damaging or modifying the structure of lipoteichoic acid through the deacylation process of its lipid components by releasing hydroxyl ions to produce an alkaline to make the acid environment so calcium hydroxide cannot stimulate the production of alpha tumour necrosis factor (TNF-α).[9,10,15]

Calcium hydroxide paste (UltraCal XS™) release a high amount of calcium and hydroxyl ions with special antimicrobial effects (UltraCal XS™) contains propylene glycol, which facilitates the solution to penetrate through the root canal. According to Grover et al.[8], propylene glycol can easily penetrate through the apical foramen, where the solution can penetrate through the dentinal tubules better than aquadest as a solvent because of its hygroscopic effects, high molecular weight (76.09), and lower surface tension also propylene glycol has an antibacterial effect against anaerobic bacteria.[12,13,51] According to Suneeth et al.[20], propylene glycol has shown a strong antibacterial action in the root canal when used as much as 20%, making it an ideal mixture and having no toxic effects on its tissues.[18]

Aquadest as a solvent for calcium hydroxide did not play an essential role in the antimicrobial effect because there was no change in the research. However, aquadest supports calcium hydroxide to pass and spread well into dentinal tubules and root canals in line with the research conducted by Grover et al.[8] that when calcium hydroxide is mixed with aquadest, calcium and hydroxyl ions will be released immediately so that it affects the pH increase in the tissue even though it does not last for a long time.[8] Furthermore, calcium hydroxide material requires a solvent in the form of aquadest because of its high solubility and easy application of calcium hydroxide medicament to pass and spread well into the tubules and root canals.[10,15]

The results showed that *E. faecalis* was more sensitive to calcium hydroxide compared to other bacteria based on the MIC value and the visual overview from the MBC results; this might be because, in this study, the method was in vitro where calcium hydroxide was in direct contact with bacteria on a 96-well microplate and bacteriological agar without any obstacle like in the real tooth such as microporosity in the dentin. In clinical conditions, it is quite difficult to use calcium hydroxide to kill *E. faecalis*. The size of *E. faecalis* is about 0.5-1 µm, while the dentinal tubules' porosity is around 4.41 µm. So they can enter the deepest part of the dentinal tubules. In addition, *E. faecalis* is a viable but non-culturable (VBNC) bacteria, which is a bacteria that can survive but not multiply. so that in this study, it is seen that the growth of sensitive *E. faecalis* is inhibited by calcium hydroxide, although its minimum killing concentration (MKC) occupies the highest position.[11,12,19]

## CONCLUSIONS

Calcium hydroxide can inhibit bacterial growth activity. *E. faecalis* (ATCC 29212) and *S. sanguinis* (ATCC 10556) are more sensitive to calcium hydroxide paste than other bacteria, with the lowest MIC and MBC on seven days of incubation since the maximum calcium and hydroxyl ions are released.

## REFERENCES

1. Rahardjo A, Maharani DA. A Review of Indonesia's Dental Health - Past, Present and Future. Int J Clin Prev Dent. 2014; 10(3): 121–6.
2. Hargreaves K, Berman L. Cohen's Pathways of the Pulp. Elsevier Saunders. 2016. 453, 573 p.
3. Aunmeungtong W, Krongbaramee T,

Khongkhunthian P. Endodontic Management of a Chronic Periapical Abscess in a Maxillary Central Incisor with an Immature Root Apex Using Platelet-Rich Fibrin: A Case Report. Eur Endod J. 2018 Oct 9;3(3):192-196. DOI: 10.14744/eej.2018.19483

4. Korona-Glowniak I, Piatek D, Fornal E, Lukowiak A, Gerasymchuk Y, Kedziora A, Bugla-Płoskonska G, Grywalska E, Bachanek T, Malm A. Patterns of Oral Microbiota in Patients with Apical Periodontitis. J Clin Med. 2021 Jun 19;10(12):2707. DOI: 10.3390/jcm10122707
5. Siqueira JF, Rôças IN. Microbiology and treatment of acute apical abscesses. Clin Microbiol Rev. 2013; 26(2): 255–73.
6. Patil S, Rao RS, Sanketh DS, Amrutha N. Microbial Flora in Oral Diseases. J Contemp Dent Pract. 2013; 14(6): 1202–8.
7. Glickman GN, Erleazer PD. Glossary of Endodontic Terms Ninth Edition. 9th ed. Chicago; 2016. 50 p.
8. Grover C, Shetty N. Evaluation of calcium ion release and change in pH on combining calcium hydroxide with different vehicles. 2019; 5(4): 434–9.
9. Sabrah AHA, Yassen GH, Gregory RL. Effectiveness of Antibiotic Medicaments against Biofilm Formation of Enterococcus faecalis and Porphyromonas gingivalis. J Endod. 2013; 39(11): 1385–9.
10. Zancan RF, Vivan RR, Milanda Lopes MR, Weckwerth PH, de Andrade FB, Ponce JB, et al. Antimicrobial Activity and Physicochemical Properties of Calcium Hydroxide Pastes Used as Intracanal Medication. J Endod. 2016; 42(12): 1822–8.
11. Dewhirst FE. The Oral Microbiome: Critical for Understanding Oral Health and Disease. J Calif Dent Assoc. 2016 Jul; 44(7): 409-10.
12. Shenoy S MK. Endodontology Enterococcus Faecalis : An Endodontic Pathogen. J Conserv Dent. 2014;7(3):11.
13. Tuon FF, Gavrilko O, Almeida S, Sumi ER, Alberto T, Rocha JL, Rosa EA. Prospective, randomised, controlled study evaluating early modification of oral microbiota following admission to the intensive care unit and oral hygiene with chlorhexidine. J Glob Antimicrob Resist. 2017 Mar;8:159-163. DOI: 10.1016/j.jgar.2016.12.007
14. Nóbrega LMM, Montagner F, Ribeiro AC, Mayer MAP GB. Molecular identification of cultivable bacteria from infected root canals associated with acute apical abscess. Braz Dent J. 2016;3(27):321.
15. Kuru S, Sepet E, İrez T, Aktaş E, Yazır Y, Duruksu G, Osmanoglu Akyol E, Ergüven M. Effects of different pulp-capping materials on cell death signaling pathways of lipoteichoic acid-stimulated human dental pulp stem cells. Odontology. 2021 Apr;109(2):547-559. DOI: 10.1007/s10266-020-00571-3
16. Shweta, Krishna P. Dental abscess : A microbiological review. Dent Res J (Isfahan). 2013;10(5):585–91.
17. Deniz Sungur, D., Aksel, H., & Purali, N. (2017). Effect of a Low Surface Tension Vehicle on the Dentinal Tubule Penetration of Calcium Hydroxide and Triple Antibiotic Paste. Journal of Endodontics, 2016;43(3);452–455.
18. Shetty S, Manjunath MK, Tejaswi S. An in-vitro evaluation of the pH change through root dentin using different calcium hydroxide preparations as an intracanal medicament. J Clin Diagnostic Res. 2014;8(10): ZC13–6.
19. Kotb RM, Elkateb MA, Ahmed AM, Kawana KY, El Meligy OA. Dentin Topographic Features following Chemomechanical Caries Removal in Primary Teeth. J Clin Pediatr Dent. 2016;40(6):472-479. DOI: 10.17796/1053-4628-40.6.472
20. Shetty S, Manjunath MK, Tejaswi S. An in-vitro evaluation of the pH change through root dentin using different calcium hydroxide preparations as an intracanal medicament. J Clin Diagnostic Res. 2014;8(10): ZC13–6.
21. Saatchi M, Shokraneh A, Navaei H, Maracy MR, Shojaei H. Antibacterial effect of calcium hydroxide combined with chlorhexidine on Enterococcus faecalis: a systematic review and meta-analysis. J Appl Oral Sci. 2014 Sep-Oct;22(5):356-65. DOI: 10.1590/1678-775720140032
22. Yen CL, Chen JH, Chien HY, Cheng JS, Lee MS, Wang YY. Using a simple spectrophotometer to analyse cypress hydrolat composition. Math Biosci Eng. 2021 Oct 21;18(6):9033-9049. DOI: 10.3934/mbe.2021445
23. Parvekar P, Palaskar J, Metgud S, Maria R, Dutta S. The minimum inhibitory

concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against Staphylococcus aureus. Biomater Investig Dent. 2020 Jul 23;7(1):105-109. DOI: 10.1080/26415275.2020.1796674

24. Estrela C, Sydney GB, Bammann LL, Felippe Júnior O. Mechanism of action of calcium and hydroxyl ions of calcium hydroxide on tissue and bacteria. Braz Dent J. 2018;6(2):85–90.