

The antibacterial efficacy of calcium hydroxide–iodophors and calcium hydroxide–barium sulfate root canal dressings on *Enterococcus faecalis* and *Porphyromonas gingivalis* in vitro

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ABSTRACT

Background: A successful endodontic treatment is inseparable from the right choice of root canal dressing. The right choice of medicaments would result in patient satisfaction. *Enterococcus faecalis* (*E. faecalis*) and *Porphyromonas gingivalis* (*P. gingivalis*) are usually found in failed root canal treatments. Calcium hydroxide is a gold standard dressing that creates an alkaline environment in the root canal and has a bactericidal effect. Commercially, there are calcium hydroxide dressings with supporting additions, including calcium hydroxide–iodophors (CH–iodophors) and Calcium hydroxide–barium sulfate (CH–barium sulfate). **Purpose:** This study aimed to compare the antibacterial efficacy between CH–iodophors and CH–barium sulfate root canal dressings on *E. faecalis* and *P. gingivalis*. **Methods:** CH–iodophors and CH–barium sulfate were obtained commercially. *E. faecalis* and *P. gingivalis* were obtained from stock culture taken from the root canal of failed endodontic treatment. *E. faecalis* and *P. gingivalis* were cultured in Petri dishes, and for each bacterium, 12 wells were made in the media. Six wells were used for the CH–iodophors group, and six wells were used for the CH–barium sulfate group. CH–iodophors and CH–barium sulfate were deployed in the wells in *E. faecalis* and *P. gingivalis* cultured media in the Petri dishes. After incubation, the inhibition zone diameters were measured. An independent t-test was used for analysis, and the significance level was set at 5%. **Results:** There is a significant difference in the antibacterial efficacy of CH–iodophors and that of CH–barium sulfate on *E. faecalis* and *P. gingivalis* ($p = 0.00001$). **Conclusion:** CH–iodophors have a higher antibacterial efficacy than CH–barium sulfate on both *E. faecalis* and *P. gingivalis*.

Keywords: *Enterococcus faecalis*; *Porphyromonas gingivalis*; calcium hydroxide; iodophors; CH–barium sulfate; patient satisfaction

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INTRODUCTION

The right choice of root canal dressing to eliminate bacteria in the root canal is important for a successful endodontic treatment. A successful endodontic treatment will result in the patient's satisfaction. Many attempts have been made to increase the success of root canal treatment, including finding efficient instrumentation, employing effective cleaning,^{1,2} using antibacterial dressings and improving irrigation materials.^{3–5}

Root canal treatment has a high success rate, but in some cases, there are failures. Isolated bacteria from root

canal treatment failures and the prevalence of these bacteria in the root canal system are caused by *Enterococcus faecalis* (*E. faecalis*) in about 45.8% to 77% of cases and by *Porphyromonas gingivalis* (*P. gingivalis*) in 28.17% of cases.⁶ Both microorganisms are among the ones that survive disinfecting protocols.⁷

E. faecalis can invade dentine tubules and spread into the peri-radicular area, which causes the formation of peri-radicular lesions after root canal treatment.⁸ *P. gingivalis* can survive in the extra-radicular region, mostly in the area approximate with the root surface and responsible for periodontitis and peri-radicular lesions.⁶ *P. gingivalis*

also contributes to root canal treatment failures through its by-product, lipopolysaccharides, which affect biological processes, inflammation and tissue destruction.⁹

Different substances have been added to calcium hydroxide to increase its efficacy. The added substances are distilled water, saline, propylene glycol, chlorhexidine, glycerine, iodophors, CH–barium sulfate, corticosteroids, antibiotics, anesthetic solution, methyl cellulose and detergents. Calcium hydroxide with added CH–barium sulfate and iodophors is commonly used in clinical practice. Iodophors have been added to calcium hydroxide to work with different bacterial characteristics.¹⁰ CH–barium sulfate is added to calcium hydroxide; aside from its antibacterial effect, this substance functions to increase radiopacity.¹¹

Root canal treatment failure may happen even after applying dressing with calcium hydroxide if the dressing is done incorrectly, depending on the right instrumentation and irrigation to remove inorganic and organic smear layers. In this research, the efficacy of adding calcium hydroxide with iodophors (CH–iodophors) and calcium hydroxide with barium sulfate (CH–barium sulfate) was analysed on *E. faecalis* and *P. gingivalis*. Based on this background, the authors would like to purposely compare the antibacterial efficacy of CH–iodophor and CH–barium sulfate root canal dressings on *E. faecalis* and *P. gingivalis*.

MATERIALS AND METHODS

This is an experimental laboratory study conducted in the Conservative Dentistry Section, Universitas Airlangga Dental Hospital and Microbiology Laboratory, Research Center, Faculty of Dental Medicine. This study was ethically approved by the Universitas Airlangga Faculty of Dental Medicine Health Research Ethical Clearance Commission (166/KKEPK.FKG). Materials used in this research were commercially available calcium hydroxide dressings with iodophors (Meta Biomed, Korea) and CH–barium sulfate (Meta Biomed, Korea). *Enterococcus faecalis* and *Porphyromonas gingivalis* bacteria used in this study were stock bacteria previously cultured from patients who failed endodontic treatment.

The method employed in this study was the agar diffusion method using Mueller–Hinton (MH) agar and Brain Heart Infusion (BHI) Broth. The method is followed according to Alharthi et al.¹² and Balouiri et al.¹³, with modifications on the sum and position of the wells in

the plates. The media were allocated for four groups of experiments. In the first group, 12 wells were prepared for CH–iodophors (six wells with *E. faecalis* and six wells with *P. gingivalis*), and in the second group, 12 wells were prepared for the CH–barium sulfate dressing (six wells with *E. faecalis* and six wells with *P. gingivalis*).

Both *E. faecalis* and *P. gingivalis* bacterial cultures from stock were moved into separate reaction tubes, each containing BHI Broth, and stirred. Incubation was done for both cultures at 37°C for 24 hours in anaerobic condition. After 24 hours, 0.5 ml of the *E. faecalis* and *P. gingivalis* bacterial cultures in BHI Broth were taken using a micropipette and poured into another reaction tube containing BHI Broth until they were equal to 0.5 McFarland standard of turbidity.

The bacterial cultures were taken from the BHI Broth using a sterile cotton swab and swabbed on the surface of each MH agar allocated for *E. faecalis* and *P. gingivalis*. An antibacterial test was conducted by making wells for the tested dressing materials (CH–iodophors and CH–barium sulfate). The samples were incubated at 37°C for 48 hours in anaerobic condition. After 48 hours, measurements were conducted on antibacterial efficacy through inhibition zone measurement using a Vernier Digital Caliper (Mitutoyo, Japan). The clear zones of inhibition around the wells were measured, revealing no bacterial growth. Data of measurements on inhibition zone diameters (in millimetres) were collected for each sample well.

Inhibition zone data were analysed statistically, and the significance level was set at 5%. SPSS 20.0 for Windows (SPSS Inc., Chicago, Illinois, USA) was used in this study. Data normality was tested using the Shapiro–Wilk test. The significance was tested using an independent t-test.

RESULTS

The number of replications (n) for each treatment group was six. Mean and standard deviations of the inhibition zone diameter of CH–iodophors and CH–barium sulfate on *E. faecalis* and *P. gingivalis* are shown in Table 1. The mean of the *E. faecalis* inhibition zone from CH–iodophors was 11.8125 mm, and the mean from CH–barium sulfate was 6.3750 mm. The mean of the *P. gingivalis* inhibition zone from CH–iodophors was 12.7875 mm, and the mean from CH–barium sulfate was 6.6750 mm.

An independent t-test was used in this study to check the significance between the CH–iodophors group and the

Table 1. Mean, standard deviation, and significance from the inhibition zone diameter of CH–iodophors and CH–barium sulfate on *E. faecalis* and *P. gingivalis*

Groups	n	<i>E. faecalis</i> Mean ± SD	P value	<i>P. gingivalis</i> Mean ± SD	P value
CH–iodophors	6	11.8125 ± 1.32001	0.00001*	12.7875 ± 1.34961	0.00001*
CH–barium sulfate	6	6.3750 ± 0.19494		6.6750 ± 0.51865	

Notes: n = replication; SD = standard deviation; * = statistically significant

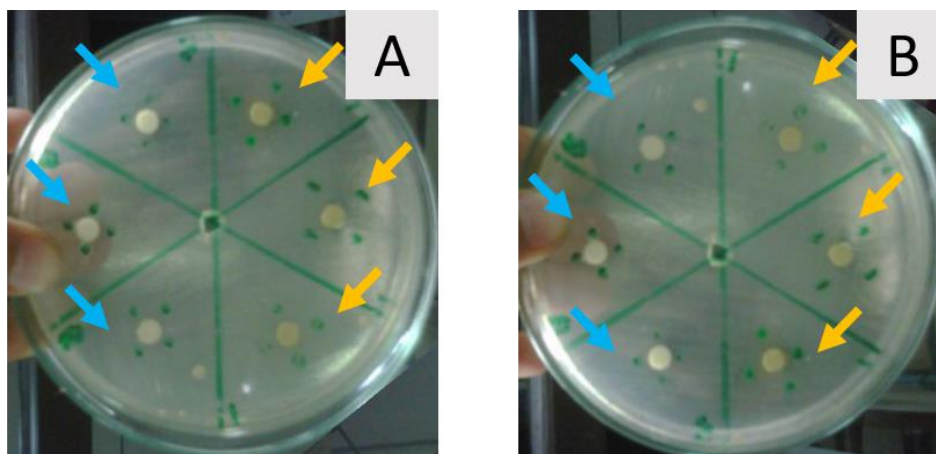


Figure 1. Agar diffusion assay containing *E. faecalis* (A) and *P. gingivalis* (B). The green dots indicate the inhibition area of each well. CH-iodophors are indicated by yellow arrows, and CH-barium sulfate is indicated by blue arrows.

CH-barium sulfate group on *E. faecalis* and *P. gingivalis*. The significance between the inhibition zone diameter of CH-iodophors and that of CH-barium sulfate on *E. faecalis* and *P. gingivalis* growth is shown in Table 1. We found a significant difference between the two treatment groups on both bacteria. The CH-iodophors group has higher antibacterial efficacy, with a wider antibacterial clear zone in both bacteria ($p = 0.00001$). The CH-barium sulfate group has lower antibacterial efficacy, with a narrower antibacterial clear zone in both bacteria. The inhibition zone diameter measurement is shown in Figure 1.

DISCUSSION

Enterococcus faecalis and *Porphyromonas gingivalis* were used in this study because these bacteria are opportunistic, living in facultative anaerobic condition, found in the pathogenic state and associated with failed root canal treatments.⁶ These bacteria also have a major role in persistent root canal infections, can survive in the root canals and are resistant to commonly used intracanal dressings.¹⁴

The antibacterial efficacy of CH-iodophors and CH-barium sulfate dressing materials on *E. faecalis* and *P. gingivalis* growths were experimentally checked with the agar diffusion method, and inhibition clear zones were measured on the media. Inhibition zone is a laboratory calculation method to check whether a material has the ability to inhibit bacterial growth.¹³ The diameter of clear zones would describe the strength of such material to inhibit bacterial growth; the more this material inhibits bacterial growth, the larger the clear zones would appear.

In this study, Mueller-Hinton agar was used because this media can grow *E. faecalis* and *P. gingivalis* actively and sensitive to drug effects. Inhibition zones appeared on both groups of CH-iodophors and CH-barium sulfate. This showed that each of these dressing materials has the ability to inhibit both *E. faecalis* and *P. gingivalis*.

The antibacterial properties of both root canal dressing materials mainly come from calcium hydroxide, a mechanism that involves the hydroxyl ion to kill bacteria by protein denaturation; the cytoplasmic membrane and DNA destruction will physically inhibit this bacterial growth in the root canal systems.¹⁵ Antibacterial activity is connected with alkali formation, which can destroy lipid, the protein structure of bacteria and nucleic acid.¹⁶ Direct contact of the hydroxyl ion in alkaline pH with a cytoplasmic membrane will destroy the hydrogen chain of the protein polypeptide. Contact of the hydroxyl ion with DNA will result in replication inhibition, causing a lethal mutation. DNA is sensitive to temperature and pH change. In alkali condition, the DNA structure and function will break and lead to bacterial cell death. The alkali condition from calcium hydroxide would impact the surrounding tissues, including healing, anti-inflammation¹⁷ and cytotoxic, leading to apoptosis.^{18,19}

The result of this study showed a significant difference between CH-iodophors and CH-barium sulfate. The ability of CH-iodophors to inhibit *E. faecalis* and *P. gingivalis* growths is significantly greater than that of the CH-barium sulfate root canal dressing. In the CH-iodophor dressing, the iodophor substance will release iodine with high reactivity to promote protein oxidation. Iodophors function as a disinfectant and infection control. Thus, in the combination of CH and iodophors, the substances will synergistically strengthen each other. Iodophors and calcium hydroxide can diffuse into dentinal tubules for added disinfection and eliminate the bacteria.

CH-barium sulfate can diffuse into dentinal tubules and also perform antibacterial activity. In this study, the antimicrobial strength of CH-barium sulfate is not as good as that of CH-iodophors. This may be caused by the form and structure of the CH-barium sulfate used in the mixture. Previous studies on the antimicrobial properties of CH-barium sulfate showed that CH-barium sulfate in micron particulate form would have potent antimicrobial properties.²⁰ CH-barium sulfate is generally used for

its radiopacity effect on radiographic examination.¹¹ The addition of this material must take into account its properties, as these will affect the dressing's consistency and application.

E. faecalis and *P. gingivalis* can be killed with high pH levels. High pH or an extreme alkaline environment would disturb the survival of most bacteria.^{21,22} However, there are some studies stating that a pH higher than 11.5 is required for potent disinfection.⁶ This study showed that the inhibition zone of CH–iodophors is two times higher than that of CH–barium sulfate. This may be caused by the combination of these two materials, since calcium hydroxide and iodophors would create a synergistic effect on the antimicrobial activity to inhibit *E. faecalis* growth. Even though this dressing material will not eliminate *E. faecalis* or *P. gingivalis*, the material is expected to weaken the bacteria, and eventually, the body's defence mechanism will be able to eliminate these bacteria and their by-products.

Much research has been done to find novel, biocompatible, antimicrobial and regenerating root canal dressing materials in order to support successful root canal treatment and enhance patient satisfaction. The success of root canal treatment, aside from the right choice of dressing, depends on other factors, such as the root canal system complexity, the diffusion ability of dressing materials within the dentinal tubules, the effect on mixed cultured bacteria or biofilm formation, and bacterial resistance, all of which need to be evaluated in further studies. As there are many microorganisms involved in failed root canal treatments,^{23–25} even after biomechanical and chemical instrumentations during treatment, individual and personalised assessments of these bacteria or biofilms are needed for ideal treatment. There are limitations, as this is only an in vitro study, and there are many factors to consider, both in situ and in vivo. In conclusion, a calcium hydroxide–iodophors root canal dressing has a different (higher) antimicrobial efficacy on both *E. faecalis* and *P. gingivalis*. However, further studies need to be done regarding the effect on periodontal and periapical tissues with more complexities.

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REFERENCES

1. Santoso CMA, Samadi K, Prasetyo EP, Wahjuningrum DA. The differences between mangosteen peel extract irrigant and NaOCl 2.5% on root canal cleanliness. *Conserv Dent J.* 2020; 10(1): 40–3.
2. Juniarti DE, Kusumaningsih T, Soetoyo A, Sunur YK. Antibacterial activity and phytochemical analysis of ethanolic purple leaf extract

- (Graptophyllum Pictum L.griff) on Lactobacillus Acidophilus. *Malaysian J Med Heal Sci.* 2021; 17(Supp 2): 71–3.
3. Astuti RHN, Samadi K, Prasetyo EP. Antibacterial activity of Averrhoa bilimbi linn leaf extract against Enterococcus faecalis. *Conserv Dent J.* 2016; 6(2): 93–8.
 4. Prasetyo EP, Saraswati W, Wahjuningrum DA, Mooduto L, Rosidin RF, Tjendronegoro E. White pomegranate (Punica granatum) peels extract bactericidal potency on Enterococcus faecalis. *Conserv Dent J.* 2021; 11(2): 84–8.
 5. Harseno S, Mooduto L, Prasetyo EP. Antibacterial potency of kedondong bangkok leaves extract (Spondias dulcis Forst.) against Enterococcus faecalis bacteria. *Conserv Dent J.* 2016; 6(2): 110–6.
 6. Prada I, Micó-Muñoz P, Giner-Lluesma T, Micó-Martínez P, Collado-Castellano N, Manzano-Saiz A. Influence of microbiology on endodontic failure. Literature review. *Med Oral Patol Oral Cir Bucal.* 2019; 24(3): e364–72.
 7. Del Fabbro M, Samaranayake LP, Loloto A, Weinstein T, Taschieri S. Analysis of the secondary endodontic lesions focusing on the extraradicular microorganisms: an overview. *J Investig Clin Dent.* 2014; 5(4): 245–54.
 8. Kaiwar A, Nadig G, Hegde J, Lekha S. Assessment of antimicrobial activity of endodontic sealers on Enterococcus faecalis: An in vitro study. *World J Dent.* 2012; 3(1): 26–31.
 9. Kuntjoro M, Prasetyo EP, Cahyani F, Kamadjaja MJK, Hendrijantini N, Laksono H, Rahmania PN, Ariestania V, Nugraha AP, Ihsan IS, Dinaryanti A, Rantam FA. Lipopolysaccharide's cytotoxicity on human umbilical cord mesenchymal stem cells. *Pesqui Bras Odontopediatria Clin Integr.* 2020; 20: e0048.
 10. Najjar RS, Alamoudi NM, El-Housseiny AA, Al Tuwriqi AA, Sabbagh HJ. A comparison of calcium hydroxide/iodoform paste and zinc oxide eugenol as root filling materials for pulpctomy in primary teeth: A systematic review and meta-analysis. *Clin Exp Dent Res.* 2019; 5(3): 294–310.
 11. Ba-Hattab R, Al-Jamie M, Aldreib H, Alessa L, Alonazi M. Calcium hydroxide in endodontics: An overview. *Open J Stomatol.* 2016; 06(12): 274–89.
 12. ALHarthi SS, BinShabaib M, Saad AlMasoud N, Shawky HA, Aabed KF, Alomar TS, AlBrekan AB, Alfaifi AJ, Melaibari AA. Myrrh mixed with silver nanoparticles demonstrates superior antimicrobial activity against Porphyromonas gingivalis compared to myrrh and silver nanoparticles alone. *Saudi Dent J.* 2021; 33(8): 890–6.
 13. Balouiri M, Sadiki M, Ibsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal.* 2016; 6(2): 71–9.
 14. Jhamb S, Singla R, Kaur A, Sharma J, Bhushan J. An in vitro determination of antibacterial effect of silver nanoparticles gel as an intracanal medicament in combination with other medicaments against Enterococcus fecalis. *J Conserv Dent.* 2019; 22(5): 479–82.
 15. Mohammadi Z, Dummer PMH. Properties and applications of calcium hydroxide in endodontics and dental traumatology. *Int Endod J.* 2011; 44(8): 697–730.
 16. Sharma G, Ahmed HMA, Zilm PS, Rossi-Fedele G. Antimicrobial properties of calcium hydroxide dressing when used for long-term application: A systematic review. *Aust Endod J.* 2018; 44(1): 60–5.
 17. Prasetyo EP, Kuntjoro M, Cahyani F, Goeharto S, Saraswati W, Juniarti DE, Hendrijantini N, Hariyani N, Nugraha AP, Rantam FA. Calcium hydroxide upregulates interleukin-10 expression in time dependent exposure and induces osteogenic differentiation of human umbilical cord mesenchymal stem cells. *Int J Pharm Res.* 2021; 13(1): 140–5.
 18. Prasetyo EP, Widjiastuti I, Cahyani F, Kuntjoro M, Hendrijantini N, Hariyani N, Winoto ER, Nugraha AP, Goeharto S, Susilowati H, Hendrianto E, Rantam FA. Cytotoxicity of calcium hydroxide on human umbilical cord mesenchymal stem cells. *Pesqui Bras Odontopediatria Clin Integr.* 2020; 20: e0044.
 19. Prasetyo EP, Kuntjoro M, Goeharto S, Juniarti DE, Cahyani F, Hendrijantini N, Nugraha AP, Hariyani N, Rantam FA. Calcium hydroxide increases human umbilical cord mesenchymal stem cells

- expressions of apoptotic protease-activating factor-1, caspase-3 and caspase-9. *Clin Cosmet Investig Dent*. 2021; 13: 59–65.
20. Aninwene II GE, Stout D, Yang Z, Webster TJ. Nano-BaSO₄: a novel antimicrobial additive to pellethane. *Int J Nanomedicine*. 2013; 8(1): 1197–205.
 21. Jhajharia K, Mehta L, Parolia A, Shetty Kv. Biofilm in endodontics: A review. *J Int Soc Prev Community Dent*. 2015; 5(1): 1–12.
 22. Zancan RF, Vivian RR, Milanda Lopes MR, Weckwerth PH, de Andrade FB, Ponce JB, Duarte MAH. Antimicrobial activity and physicochemical properties of calcium hydroxide pastes used as intracanal medication. *J Endod*. 2016; 42(12): 1822–8.
 23. Dudeja P, Dudeja K, Srivastava D, Grover S. Microorganisms in periradicular tissues: Do they exist? A perennial controversy. *J Oral Maxillofac Pathol*. 2015; 19(3): 356–63.
 24. Pereira RS, Rodrigues VAA, Furtado WT, Gueiros S, Pereira GS, Avila-Campos MJ. Microbial analysis of root canal and periradicular lesion associated to teeth with endodontic failure. *Anaerobe*. 2017; 48: 12–8.
 25. Siqueira JF, Antunes HS, Rôças IN, Rachid CTCC, Alves FRF. Microbiome in the apical root canal system of teeth with post-treatment apical periodontitis. Rittling SR, editor. *PLoS One*. 2016; 11(9): e0162887.