eric priyo prasetyo <eric-p-p@fkg.unair.ac.id>

Submission letter

2 messages

Dental Journal (Majalah Kedokteran Gigi) <dental_journal@fkg.unair.ac.id> Fri, Feb 11, 2022 at 11:25 AM
To: eric priyo prasetyo <eric-p-p@fkg.unair.ac.id>, Eric Prasetyo <eppfkgunair@gmail.com>

Dear
Dr. Eric Priyo Prasetyo, drg.,M.Kes.,Sp.KG

Thank you for your article submission to Dental Journal (Majalah Kedokteran Gigi) and we gladly inform you that your article:

In-vitro antibacterial efficacy between calcium hydroxide-iodoform and calcium hydroxide-barium sulfate root canal dressings on *Enterococcus faecalis* and *Porphyromonas gingivalis*

Authors: Eric Priyo Prasetyo,[1] Devi Eka Juniarti,[1] Galih Sampoerno,[1] Dian Agustin Wahjuningrum,[1] Ananta Tantri Budi,[1] Dyanita Hasri, [2] Evelyn Tjendronegoro[3]

The article is under review according to the provisions of the issuance of the Dental Journal (Majalah Kedokteran Gigi).
Certainty of revision or rejection of the article will be notified approximately one month after this notification.
If the article is accepted for publication the author is subject to administrative fees as follows:

- Proofreading fees of GBP 20* per 1000 words.
(*prices can change at any time, and the value of the rupiah follows the last exchange rate at that time and does not include transfer fee).

Please find the attached cover letter and copyright form, please fill it out and sign it by all authors and send it back by email.

Thank you for considering this journal as a venue for your work.

Best Regards,
Chief Editor,

Muhammad Dimas Aditya Ari, drg., M.Kes., Sp.Pros

Dental Journal (Majalah Kedokteran Gigi)
http://e-journal.unair.ac.id/MKG

Faculty of Dental Medicine, Universitas Airlangga

Jl. Mayjen Prof. Dr. Moestopo 47 Surabaya 60132 INDONESIA
Phone/Fax: +62 31 5039478

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eric priyo prasetyo <eric-p-p@fkg.unair.ac.id> Fri, Feb 11, 2022 at 7:20 PM
To: "Dental Journal (Majalah Kedokteran Gigi)" <dental_journal@fkg.unair.ac.id>

Dear Chief Editor,

Thank you for your email regarding our manuscript submission,
Hereby we attach the cover letter and copyright transfer form in the attachment.

Thank you once again.

Best regards,
Dr. Eric Priyo Prasetyo, drg., M.Kes., Sp.KG(K). & team.
[Quoted text hidden]

Cover_letter_n_copyright_form Eric CHI CHBS EF PG.pdf
227K

eric priyo prasetyo <eric-p-p@fkg.unair.ac.id>

Editor decision: revision required

3 messages

Dental Journal (Majalah Kedokteran Gigi) <dental_journal@fkg.unair.ac.id> Tue, Feb 22, 2022 at 9:53 AM
To: eric priyo prasetyo <eric-p-p@fkg.unair.ac.id>, Eric Prasetyo <eppfkgunair@gmail.com>

Dear
Dr. Eric Priyo Prasetyo,drg.,M.Kes.,Sp.KG(K)

We have reached a decision regarding your submission to Dental Journal (Majalah Kedokteran Gigi),

In-vitro antibacterial efficacy between calcium hydroxide-iodoform and calcium hydroxide-barium sulfate root canal dressings on Enterococcus faecalis and Porphyromonas gingivalis
Our decision is to: **Revise your manuscript**

Please revise according to the reviewer comments, highlight the text with color on the changes made and make a response letter (attached).
Please send the revised manuscript within a week (**3 March 2022**).

Determination of acceptance of the manuscript based on the revised results sent.

Best Regards,
Chief Editor,

Muhammad Dimas Aditya Ari, drg., M.Kes., Sp.Pros

Dental Journal (Majalah Kedokteran Gigi)
http://e-journal.unair.ac.id/MKG

Faculty of Dental Medicine, Universitas Airlangga
Jl. Mayjen Prof. Dr. Moestopo 47 Surabaya 60132 INDONESIA
Phone/Fax: +62 31 5039478

4 attachments

Response letter.docx
22K

review 1 Eric Priyo article_In-vitro antibacterial efficacy between calcium (PA2).pdf
160K

review 1 Eric Priyo article_In-vitro antibacterial efficacy between calcium (PA1)-.pdf
622K

review 1 Eric Priyo article_In-vitro antibacterial efficacy between calcium (PP)-.pdf
553K

eric priyo prasetyo <eric-p-p@fkg.unair.ac.id> Fri, Feb 25, 2022 at 6:24 PM

To: "Dental Journal (Majalah Kedokteran Gigi)" <dental_journal@fkg.unair.ac.id>

Dear Dr. Muhammad Dimas Aditya Ari,

Thank you for your decision about the revisions for our manuscript.
Please find the revised manuscript and response letter in the attachment.
We hope the revisions will satisfy the reviewers and managing editor.
Thank you once again for your consideration.

Best regards,
Dr. Eric & team.

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2 attachments

[DJMKG] Original article template CHI CHBS EF PG Eric et al Rev 1.docx
689K

Response letter review Eric et al.pdf
240K

Dental Journal (Majalah Kedokteran Gigi) <dental_journal@fkg.unair.ac.id> Tue, Mar 1, 2022 at 8:47 AM
To: eric priyo prasetyo <eric-p-p@fkg.unair.ac.id>

Dear
Dr. Eric Priyo Prasetyo,drg.,M.Kes.,Sp.KG(K)

Thank you for submitting a revised draft of your manuscript.
The manuscript will be re-evaluated by reviewers based on the revisions you make.

Best wishes,
Dental Journal (Majalah Kedokteran Gigi)
http://e-journal.unair.ac.id/MKG

Faculty of Dental Medicine, Universitas Airlangga
Jl. Mayjen Prof. Dr. Moestopo 47 Surabaya 60132 INDONESIA
Phone/Fax: +62 31 5039478

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ASSESSMENT FORM OF ORIGINAL ARTICLE DENTAL JOURNAL (MAJALAH KEDOKTERAN GIGI)

Manuscript title: **In-vitro antibacterial efficacy between calcium hydroxide-iodoform and calcium hydroxide-barium sulfate root canal dressings on *Enterococcus faecalis* and *Porphyromonas gingivalis***

Send date: Receive date:

Please comments written in English

REVIEW	YES*	NO*
1. Has this text ever been published on other media? Comment: This text ever been published on other media but, there have been several studies to test the antibacterial efficacy of calcium hydroxide-iodoform for root canal dressing against Enterococcus bacteria		V
2. Is the title appropriate, concise, clear, and describes the contribution of scientific development? (maximum 10 words, covering the variables studied) Comment: The tithe too long, please make the short title likes this : Calcium hydroxide-iodoform and calcium hydroxide-barium sulfate antibacterial efficacy for root canal dressings.		V
3. Research report:		
a) Introduction includes the background clearly? Comment: Please add the more clear function of iodoform and barium sulfate to increase the antibacterial efficacy	V	
b) The purpose clearly? Comment:The purpose is clear	V	
c) Methods and research design in accordance with the purpose of the study? Comment: Please write the spesific nomenclature/strain of E.faecalis and P gingivalis (E.faecalis ATCC……, P.gingiivalis ATCC……) that use in this research.	V	
d) The research procedure is described precisely and in detail, thus ensuring internal/external validity? Comment: The procedure is clear and detail	V	
e) The results can answer the research question? Comment: The result can answer the research question	V	
f) - The discussion does not repeat the results?		V

- Aligned with the scope of the study and compared with similar research results? - Explain the meaning of research results in answering the problem? Comment: 1. There are several statement not necessary in discussion, i.e in paragraf 1 and 3. 2. Please compare this research with similar research result.		
g) References are aligned with the research material and use the literature of the last 10 years? Comment: References aligned with the research material, but references number 23 : use journal at 2002 years.		V
h) - The conclusion matches the title and the problem? - The research results contibute to the development of dentistry? - Perform synthesis base on similar research results that precede? Comment: Conclusions should be adjusted to the title and research objectives, the title mentions the difference but at the conclusion only mentions that calcium hydroxide-iodoform has a higher antimicrobial efficacy.	V	
i) References needs to be added/substacted**? Comment: References are already enough but lthe old literature should be change		
4. Is there a section that needs to be added/summarized**? Comment: No.		

Note:
1. *) Put a check mark (√), **) Cross the unnecessary ones
2. Correction can be made directly on the script
3. If the inquiry form is lacking, please write on the additional sheet

Recommendation for Editors

[.....] 1. The script can be published without changes.
[V] 2. The manuscript can be published with corrections according to the direction of the Reviewer (suggestions for improvement please write direcly to the script).
Comment: ……..
[……..] 3. The manuscript could not be published.
Reason: ……..

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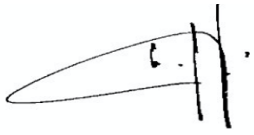
Date: Pebruary. 21, 2022

Reviewer,



ASSESSMENT FORM OF ORIGINAL ARTICLE DENTAL JOURNAL (MAJALAH KEDOKTERAN GIGI)

Manuscript title: **In-vitro antibacterial efficacy between calcium hydroxide-iodoform and calcium hydroxide-barium sulfate root canal dressings on *Enterococcus faecalis* and *Porphyromonas gingivalis***

Send date:21thFebruary 2022 Receive date: 11thFebruary 2022

Please comments written in English

REVIEW	YES*	NO*
1. Has this text ever been published on other media? Comment: This text never been published on other media		√
2. Is the title appropriate, concise, clear, and describes the contribution of scientific development? (maximum 10 words, covering the variables studied) Comment: ..... The title is appropriate, concise, cler and describes the contribution of scientific development	√	
3. Research report:		
a) Introduction includes the background clearly? Comment: Introduction includes the background clearly	√	
b) The purpose clearly? Comment: The purpose is clear	√	
c) Methods and research design in accordance with the purpose of the study? Comment: The methods and research design has been appropiate with the purpose of the study	√	
d) The research procedure is described precisely and in detail, thus ensuring internal/external validity? Comment: The research procedure has described precisely, and covers internal/external validity	√	
e) The results can answer the research question? Comment:	√	

The results has answer the research question, but need described more for clinical needs		
f) - The discussion does not repeat the results? - Aligned with the scope of the study and compared with similar research results? - Explain the meaning of research results in answering the problem? Comment: The discussion does not repeat the results, has been aligned with the scope of the study and has been compared with similar research results The discussion has explained the meaning of reseach results in answering the problem	√	
g) References are aligned with the research material and use the literature of the last 10 years? Comment: References are aligned with the research material and use the literature of the last 10 years	√	
h) - The conclusion matches the title and the problem? - The research results contibute to the development of dentistry? - Perform synthesis base on similar research results that precede? Comment: ..... The conclusion has matched the title and the problem, but need more description for clinical needs ..... The research results can contribute to the development of dentistry, espescially endodontic, but need further research , both in situ and in vivo research ..... The conclusion has perform synthesis base on similar research results that precede.....	√	
i) References needs to be added/substacted**? Comment: The references no need to be added/substacted		
4. Is there a section that needs to be added/summarized**? Comment: There is a section that needs to be added		

Note:
1. *) Put a check mark (√), **) Cross the unnecessary ones
2. Correction can be made directly on the script
3. If the inquiry form is lacking, please write on the additional sheet

Recommendation for Editors

[.....] 1. The script can be published without changes.

[....√.....] 2. The manuscript can be published with corrections according to the direction of the Reviewer (suggestions for improvement please write direcly to the script).

Comment: ……..……..

.Please see to manuscript.....

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[……..] 3. The manuscript could not be published.

Reason: ……..……..

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. Date: .20th February 2020

Reviewer,

Original article

In-vitro antibacterial efficacy between calcium hydroxide-iodoform and calcium hydroxide-barium sulfate root canal dressings on *Enterococcus faecalis* and *Porphyromonas gingivalis*

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 which create an alkaline environment in the root canal and have a bactericidal effect. Commercially, there are calcium hydroxide dressings with supporting additions, including calcium hydroxide-iodoform (CH-Iodoform) and Calcium hydroxide-barium sulfate (CH-Barium Sulfate).* ***Purpose:*** *This study aimed to determine the antibacterial efficacy of CH-Iodoform and CH-Barium Sulfate on E. faecalis and P. gingivalis.* ***Methods:*** *CH-Iodoform and CH-Barium Sulfate was 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, 6 wells were used for CH-Iodoform group and 6 wells were used for CH-Barium Sulfate group. CH-Iodoform and CH-Barium Sulfate were deployed in the wells in E. faecalis and P. gingivalis cultured medium in the petri dishes. After incubation, the inhibition zone diameters were measured. Independent t-test was used for analysis and significance level was set at 5%.* ***Results****: There is a significant antibacterial efficacy between CH-Iodoform and CH-Barium Sulfate on E. faecalis and P. gingivalis (p=0.00001).* ***Conclusion:*** *CH-Iodoform has a better antibacterial efficacy than CH-Barium Sulfate on both E. faecalis and P. gingivalis.*

Keywords: *Enterococcus faecalis; Porphyromonas gingivalis; calcium hydroxide; iodoform; barium sulfate; patient satisfaction.*

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 would result in patient's satisfaction. Many attempts were conducted to increase the success of root canal treatment, including finding the efficient instrumentation, effective cleaning,[1,2] antibacterial dressing and irrigation materials.[3,4]

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 are about 45.8-77% caused by *E. faecalis* and 28.17% caused by *P. gingivalis* in the root canal system.[5] Both microorganisms are among the ones that survive disinfecting protocol.[6]

E. faecalis can invade dentine tubules and ~~rsistence and periapical abnormalities which occur several months~~ ~~or years~~ after root canal treatment.[7] *P. gingivalis* can survive in the extra-radicular area mostly in the area approximate with root surface, and responsible for periodontitis and peri-radicular lesions.[8] *P. gingivalis* also contribute to root canal treatment failures, through its byproduct, lipopolysaccharide which effect 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, glycerin, iodoform, barium sulfate, corticosteroids, antibiotics, anesthetic solution, methyl cellulose, and detergents. Iodoform has been added to calcium hydroxide to work with different bacterial characteristic.[10] Barium sulfate is added to calcium hydroxide aside from its antibacterial effect, it functions to increase radiopacity.[11]

Root canal treatment failure may happen even after dressing with calcium hydroxide. In this research, the efficacy of calcium hydroxide addition with iodoform (CH-Iodoform) and barium sulfate (CH-Barium Sulfate) was analyzed on *E. faecalis* and *P. gingivalis.* Based on this background, the authors would like to purposely compare the antibacterial efficacy between CH-Iodoform and CH-Barium Sulfate root canal dressing on *E. faecalis* and *P. gingivalis*.

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MATERIALS AND METHODS

This is an experimental laboratory study, conducted in Conservative Dentistry Section, Universitas Airlangga Dental Hospital and Microbiology Laboratory, Research Center, Faculty of Dental Medicine, Universitas Airlangga. Materials used in this research were commercially available calcium hydroxide dressings with Iodoform (Meta Biomed, Korea) and Barium Sulfate (Meta Biomed, Korea). *Enterococcus faecalis* and *Porphyromonas gingivalis* bacteria used in this study were stock bacteria previously cultured from failed endodontic treatment.

The method used in this study was agar diffusion method using Mueller Hinton (MH) agar and Brain Heart Infusion (BHI) broth. The method is according to Alharthi et al. (2021) and Balouiri et al. (2016) with modifications.[12,13] The media were allocated for 4 groups of experiments. In the first group, 12 wells were prepared for CH-Iodoform (6 wells with *E. faecalis* and 6 wells with *P. gingivalis*), and in the second group, 12 wells were prepared for CH-Barium Sulfate dressing (6 wells with *E. faecalis* and 6 wells with *P. gingivalis*).

Both *E. faecalis* and *P. gingivalis* bacterial culture from stock was moved into separate reaction tube, each containing BHI broth and stirred. Incubation was done for both culture at 37˚C for 24 hours in anaerobic condition. After 24 hours, *E. faecalis* and *P. gingivalis* bacterial culture in BHI broth was taken 0.5 ml using micro pipette, and poured into another reaction tube containing BHI broth until equal with 0.5 McFarland standard of turbidity.

The bacterial cultures were taken from BHI broth using sterile cotton swab and swabbed on the surface of each MH agar allocated for *E. faecalis* and *P gingivalis.* Antibacterial test was conducted by making the wells for the tested dressing materials (CH-Iodoform 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 millimeters) were collected for each sample wells.

Inhibition zones data were analyzed statistically and significance level was set at 5%. Data normality was tested using Shapiro Wilk test. The significance was tested using independent t-test.

Commented [Ta6]: ...from patients who failed endodotic treatment

Commented [Ta7]: This Method just for *P. gingivalis*

Commented [Ta8]: ..need explain how the modification is conducted

RESULTS

The number of replications (n) for each treatment group was 6. Mean and standard deviation of inhibition zone diameter of CH-Iodoform and CH-Barium Sulfate on *E. faecalis* and *P. gingivalis* are shown in Table 1. The mean of *E. faecalis* inhibition zone from CH-Iodoform was 11.8125 mm and CH-Barium Sulfate was 6.3750 mm. The mean of *P. gingivalis* inhibition zone from CH-Iodoform was 12.7875 mm and CH-Barium Sulfate was 6.6750 mm.

Table 1:

Mean and standard deviation from inhibition zone diameter of CH-Iodoform and CH-Barium Sulfate on *E. faecalis* and *P. gingivalis*

Groups	**n**	***E. faecalis*** **Mean ± SD**	***P. gingivalis*** **Mean ± SD**
CH-Iodoform	6	11.8125 ± 1.32001	12.7875 ± 1.34961
CH-Barium Sulfate	6	6.3750 ± 0.19494	6.6750 ± 0.51865

n = replication

SD = standard deviation

Table 2: Significance between inhibition zone diameter of CH-Iodoform and CH-Barium Sulfate on *E. faecalis* and *P. gingivalis*

Groups	***E. faecalis*** **CH-Iodoform**	***P. gingivalis*** **CH-Iodoform**
E. faecalis **CH-Barium Sulfate**	0.00001*	-
P. gingivalis **CH-Barium Sulfate**	-	0.00001*

*Statistically significant

Independent t-test was used in this study to check the significance between CH-Iodoform group and CH-Barium Sulfate group on *E. faecalis* and *P. gingivalis*. The significance between inhibition zone diameter of CH-Iodoform and CH-Barium Sulfate on *E. faecalis* and *P. gingivalis* growth is shown in Table 2. We found a significant difference between the two treatment groups on both bacteria.

The CH-Iodoform group has higher antibacterial efficacy with wider antibacterial clear zone in both bacteria ($p = 0.00001$). CH-Barium Sulfate group has lower antibacterial efficacy with narrower antibacterial clear zone in both bacteria. There is no significant difference of antibacterial activity of CH-Iodoform between *E. faecalis* and *P. gingivalis* ($p = 0.11726$). There is also no significant difference of antibacterial activity of CH-Barium Sulfate between *E. faecalis* and *P. gingivalis* ($p = 0.10712$).

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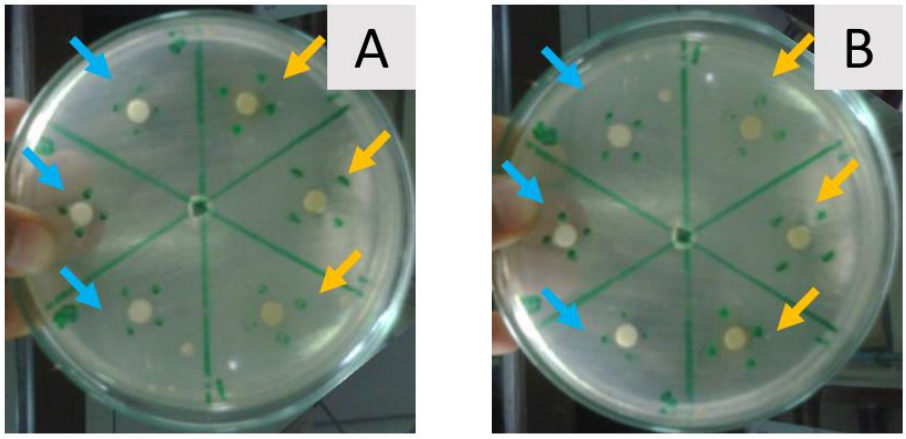

Figure 1.

Agar diffusion assay containing *E. faecalis* (A) and *P. gingivalis* (B). The green dots indicate the inhibition area of each well. CH-Iodoform is pointed by yellow arrows, and CH-Barium Sulfate is pointed by blue arrows.

DISCUSSION

Enterococcus faecalis and *Porphyromonas gingivalis* were used in this study because these bacteria are opportunistic, living in facultative anaerobic condition, found in pathogenic state, and associated with failed root canal treatment.[5] These bacteria also have a major role in persistent root canal infections, can survive in the root canals and resistant to commonly used intracanal dressings.[14]

Antibacterial efficacy of CH-Iodoform and CH-Barium Sulfate dressing materials on *E. faecalis* and *P. gingivalis* growths were experimentally checked with agar diffusion method and inhibition clear zone were measured on the media. Inhibition zone is a laboratory calculation method to check whether a material have the ability to inhibit bacterial growth.[13] The diameter of clear zones would describe the strength of such material to inhibit bacterial growth, the stronger this material inhibit, 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 drugs effect. Inhibition zones appeared on both groups of CH-Iodoform and CH-Barium Sulfate. This showed that each of these dressing materials have the ability to inhibit both *E. faecalis* and *P. gingivalis*.

The antibacterial properties of both root canal dressing materials mainly come from calcium hydroxide, which mechanism involve hydroxyl ion to kill bacteria by protein denaturation, 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 destruct lipid, the protein structure of bacteria and nucleic acid.[16] Direct contact of hydroxyl ion in alkaline pH with cytoplasmic membrane will destruct the hydrogen chain of protein polypeptide. Contact of hydroxyl ion with DNA will result in replication inhibition, causing lethal mutation. DNA is sensitive to temperature and pH change. In alkali condition DNA structure and function will break and leading to bacterial cell death. Alkali condition from calcium hydroxide would impact on the surrounding tissues, including healing, anti-inflammation[17] and cytotoxic which lead to apoptosis.[18,19]

The result of this study showed a significant difference between CH-Iodoform and CH-Barium Sulfate. The ability of CH-Iodoform to inhibit *E. faecalis* and *P. gingivalis* growths are significantly greater than CH-Barium Sulfate root canal dressing. In CH-Iodoform dressing, the iodoform substance will release iodine with high reactivity to promote protein oxidation. Iodoform functions as disinfectant and infection control. Iodoform and calcium hydroxide can diffuse into dentinal tubules for added disinfection and ~~eventually will stimulate tissue repair~~.

Commented [Ta10]: It is clear CH-Iodoform to inhibit E.faecalis and P. gingivalis because CH with ions OH can change the surrounding tissue to alkali conditions, Iodoform will release iodine with high reactivity to promote protein oxidation. Thus the combination CH and Iodoform will strengthen each other.

Commented [Ta11]: …and eliminate the bacteri

In CH-Barium Sulfate, barium sulfate can diffuse into dentinal tubule and also perform antibacterial activity. In this study the antimicrobial strength is not as good as CH-Iodoform. This may be caused by the form and structure of barium sulfate used in the mixture. Previous study on antimicrobial of barium sulfate showed that barium sulfate in micron particulate form would have potent antimicrobial properties.[20] Barium sulfate is generally used for its radiopacity effect on radiographic examination.[11]

E. faecalis and *P. gingivalis* can be killed with high pH level. High pH or an extreme alkaline environment would disturb the survival of most bacteria.[21,22] However, there are some studies stated that pH of higher than 11.5 is required for potent disinfection.[23] This study showed inhibition zone of CH-Iodoform is two folds higher than CH-Barium Sulfate, this may be caused by the combination of these two materials, calcium hydroxide and iodoform would create synergistic effect on the antimicrobial activity to inhibit *E. faecalis* growth. Even though this dressing material won't eliminate *E. faecalis or P. gingivalis*, the material is expected to weaken the bacteria, and eventually body defense mechanism will be able eliminate these bacteria and their byproducts.

Many researches have been done to found novel, biocompatible, antimicrobial and regenerating root canal dressing materials in order to support successful root canal treatment and enhance patient's satisfaction. The success of root canal treatment aside from the right choice of dressing, there are other factors such as the root canal systems complexity, diffusion ability of dressing materials within the dentinal tubules, and the effect on mixed cultured bacteria or biofilm formation, and bacterial resistance, which need to be evaluated in further studies. ~~As there are many microorganisms involved in failed root canal treatments,[24-26] even after biomechanical and chemical instrumentations during treatment, individual and personalized assessment of these bacteria or biofilm is needed for ideal treatment.~~ In conclusion, calcium hydroxide-Iodoform root canal dressing has 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.

Commented [Ta12]: If we add the barium sulfat to CH, must be considered how the nature of the material as the flow rate etc

Commented [Ta13]: The limitations of this research just done as in vitro study. There are many factors to consider if used the material directly on teeth, both in situ and in vivo study.

ACKNOWLEDGEMENT

The authors would like to thank the Publication Center, Dental Hospital, and Research Center, Faculty of Dental Medicine, Universitas Airlangga for the given facility.

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Manuscript Title: **In-vitro antibacterial efficacy between calcium hydroxide-iodoform and calcium hydroxide-barium sulfate root canal dressings on *Enterococcus faecalis* and *Porphyromonas gingivalis***

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Please comments written in English

REVIEW	COMMENT*
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▪ Sections of the contens on the script are proportional (The discussion is longer than the introduction	Yes
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▪ In accordance with the problem, purpose and contain the main variable	Yes/
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▪ Keywords must be provided 3-5 words and/or a phrase and separated by a semicolon (;)	No
▪ The Abstract in Research Reports should consist of a single paragraph containing **Background:**, **Purpose:**, **Methods:**, **Results:** and **Conclusion:** written in bold type	Yes
References	
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Figures and Tables	
▪ In accordance with the style of the journal (the truth, the completeness of the title and description/ legend) and included reference	No
▪ Number of figure/ tables in the research report and literature review maximum 4	No
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References (*Vancouver superscript*)	
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ORIGINAL ARTICLE	
FORMAT	
▪ Systematic manuscript of the original research consists of the introduction, materials and methods, results, discussion ended conclusion, and references.	Yes
Introduction	
▪ Empirical/ theoretical background	There is
▪ Problems/ goals	There is
▪ Purpose of the study	There is
Materials and Methods	
▪ Design (type, time, place of the study)	No
▪ Sampling technique	No
▪ How research works	No

▪ Data analysis	No
Results	
▪ Exposure data	No
▪ Analyze results	No
Discussion	
▪ Discussion does not repeat the results	No
▪ Aligned with the scope of the study and compared with similar research results?	No
▪ Explain the meaning of research results and answer the problem	Yes
▪ Conclusion	There is
▪ Suggesstions	No

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1. *) Cross the unnecessary ones.
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Original article

~~In vitro~~ antibacterial efficacy between calcium hydroxide-iodoform and calcium hydroxide-barium sulfate root canal dressings on *Enterococcus faecalis* and *Porphyromonas gingivalis* *in vitro*

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 which create an alkaline environment in the root canal and have a bactericidal effect. Commercially, there are calcium hydroxide dressings with supporting additions, including calcium hydroxide-iodoform (CH-Iodoform) and Calcium hydroxide-barium sulfate (CH-Barium Sulfate).* ***Purpose:*** *This study aimed to determine the antibacterial efficacy of CH-Iodoform and CH-Barium Sulfate on E. faecalis and P. gingivalis.* ***Methods:*** *CH-Iodoform and CH-Barium Sulfate was 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, 6 wells were used for CH-Iodoform group and 6 wells were used for CH-Barium Sulfate group. CH-Iodoform and CH-Barium Sulfate were deployed in the wells in E. faecalis and P. gingivalis cultured medium in the petri dishes. After incubation, the inhibition zone diameters were measured. Independent t-test was used for analysis and significance level was set at 5%.* ***Results****: There is a significant antibacterial efficacy between CH-Iodoform and CH-Barium Sulfate on E. faecalis and P. gingivalis (p=0.00001; p<0.05).* ***Conclusion:*** *CH-Iodoform has a better antibacterial efficacy than CH-Barium Sulfate on both E. faecalis and P. gingivalis.*

Keywords: *Enterococcus faecalis; Porphyromonas gingivalis; calcium hydroxide; iodoform; barium sulfate; patient satisfaction.*

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 would

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result in patient's satisfaction. Many attempts were conducted to increase the success of root canal treatment, including finding the efficient instrumentation, effective cleaning,[1,2] antibacterial dressing and irrigation materials.[3,4]

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 are about 45.8-77% caused by *E. faecalis* and 28.17% caused by *P. gingivalis* in the root canal system.[5] Both microorganisms are among the ones that survive disinfecting protocol.[6]

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E. faecalis can invade dentine tubules and unreachable from chemo-mechanical preparation and dressings. This may cause unseen persistence and periapical abnormalities which occur several months or years after root canal treatment.[7] *P. gingivalis* can survive in the extra-radicular area mostly in the area approximate with root surface, and responsible for periodontitis and peri-radicular lesions.[8] *P. gingivalis* also contribute to root canal treatment failures, through its byproduct, lipopolysaccharide which effect 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, glycerin, iodoform, barium sulfate, corticosteroids, antibiotics, anesthetic solution, methyl cellulose, and detergents. Iodoform has been added to calcium hydroxide to work with different bacterial characteristic.[10] Barium sulfate is added to calcium hydroxide aside from its antibacterial effect, it functions to increase radiopacity.[11]

Root canal treatment failure may happen even after dressing with calcium hydroxide. In this research, the efficacy of calcium hydroxide addition with iodoform (CH-Iodoform) and barium sulfate (CH-Barium Sulfate) was analyzed on *E. faecalis* and *P. gingivalis.* Based on this background, the authors would like to purposely compare the antibacterial efficacy between CH-Iodoform and CH-Barium Sulfate root canal dressing on *E. faecalis* and *P. gingivalis*.

MATERIALS AND METHODS

This is an experimental laboratory study, conducted in Conservative Dentistry Section, Universitas Airlangga Dental Hospital and Microbiology Laboratory, Research Center, Faculty of Dental Medicine, Universitas Airlangga. Materials used in this research were commercially available calcium hydroxide dressings with Iodoform (Meta Biomed, Korea) and Barium Sulfate (Meta Biomed, Korea). *Enterococcus faecalis* and *Porphyromonas gingivalis* bacteria used in this study were stock bacteria previously cultured from failed endodontic treatment.

The method used in this study was agar diffusion method using Mueller Hinton (MH) agar and Brain Heart Infusion (BHI) broth. The method is according to Alharthi et al. (2021) and Balouiri et al. (2016) with modifications.[12,13] The media were allocated for 4 groups of experiments. In the first group, 12 wells were prepared for CH-Iodoform (6 wells with *E. faecalis* and 6 wells with *P. gingivalis*), and in the second group, 12 wells were prepared for CH-Barium Sulfate dressing (6 wells with *E. faecalis* and 6 wells with *P. gingivalis*).

Both *E. faecalis* and *P. gingivalis* bacterial culture from stock was moved into separate reaction tube, each containing BHI broth and stirred. Incubation was done for both culture at 37˚C for 24 hours in anaerobic condition. After 24 hours, *E. faecalis* and *P. gingivalis* bacterial culture in BHI broth was taken 0.5 ml using micro pipette, and poured into another reaction tube containing BHI broth until equal with 0.5 McFarland standard of turbidity.

The bacterial cultures were taken from BHI broth using sterile cotton swab and swabbed on the surface of each MH agar allocated for *E. faecalis* and *P gingivalis.* Antibacterial test was conducted by making the wells for the tested dressing materials (CH-Iodoform 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 millimeters) were collected for each sample wells.

Inhibition zones data were analyzed statistically and significance level was set at 5%. Data normality was tested using Shapiro Wilk test. The significance was tested using independent t-test.

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RESULTS

The number of replications (n) for each treatment group was 6. Mean and standard deviation of inhibition zone diameter of CH-Iodoform and CH-Barium Sulfate on *E. faecalis* and *P. gingivalis* are shown in Table 1. The mean of *E. faecalis* inhibition zone from CH-Iodoform was 11.8125 mm and CH-Barium Sulfate was 6.3750 mm. The mean of *P. gingivalis* inhibition zone from CH-Iodoform was 12.7875 mm and CH-Barium Sulfate was 6.6750 mm.

Table 1:

Mean and standard deviation from inhibition zone diameter of CH-Iodoform and CH-Barium Sulfate on *E. faecalis* and *P. gingivalis*

Groups	**n**	***E. faecalis*** **Mean ± SD**	***P. gingivalis*** **Mean ± SD**
CH-Iodoform	6	11.8125 ± 1.32001	12.7875 ± 1.34961
CH-Barium Sulfate	6	6.3750 ± 0.19494	6.6750 ± 0.51865

n = replication

SD = standard deviation

Table 2: Significance between inhibition zone diameter of CH-Iodoform and CH-Barium Sulfate on *E. faecalis* and *P. gingivalis*

Groups	***E. faecalis*** **CH-Iodoform**	***P. gingivalis*** **CH-Iodoform**
E. faecalis **CH-Barium Sulfate**	0.00001*	-
P. gingivalis **CH-Barium Sulfate**	-	0.00001*

*Statistically significant

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Independent t-test was used in this study to check the significance between CH-Iodoform group and CH-Barium Sulfate group on *E. faecalis* and *P. gingivalis*. The significance between inhibition zone diameter of CH-Iodoform and CH-Barium Sulfate on *E. faecalis* and *P. gingivalis* growth is shown in Table 2. We found a significant difference between the two treatment groups on both bacteria.

The CH-Iodoform group has higher antibacterial efficacy with wider antibacterial clear zone in both bacteria ($p = 0.00001$; $p<0.05$). CH-Barium Sulfate group has lower antibacterial efficacy with narrower antibacterial clear zone in both bacteria. There is no significant difference of antibacterial activity of CH-Iodoform between *E. faecalis* and *P. gingivalis* ($p = 0.11726$;$p<0.05$). There is also no significant difference of antibacterial activity of CH-Barium Sulfate between *E. faecalis* and *P. gingivalis* ($p = 0.10712$;$p<0.05$).

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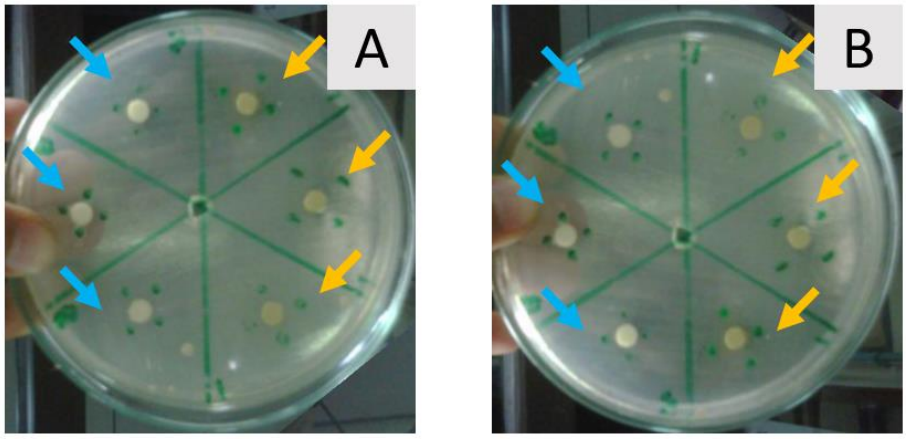

Figure 1.

Agar diffusion assay containing *E. faecalis* (A) and *P. gingivalis* (B). The green dots indicate the inhibition area of each well. CH-Iodoform is pointed by yellow arrows, and CH-Barium Sulfate is pointed by blue arrows.

DISCUSSION

Enterococcus faecalis and *Porphyromonas gingivalis* were used in this study because these bacteria are opportunistic, living in facultative anaerobic condition, found in pathogenic state, and associated with failed root canal treatment.[5] These bacteria also have a major role in persistent root canal infections, can survive in the root canals and resistant to commonly used intracanal dressings.[14]

Antibacterial efficacy of CH-Iodoform and CH-Barium Sulfate dressing materials on *E. faecalis* and *P. gingivalis* growths were experimentally checked with agar diffusion method and inhibition clear zone were measured on the media. Inhibition zone is a laboratory calculation method to check whether a material have the ability to inhibit bacterial growth.[13] The diameter of clear zones would describe the strength of such material to inhibit bacterial growth, the stronger this material inhibit, 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 drugs effect. Inhibition zones appeared on both groups of CH-Iodoform and CH-Barium Sulfate. This showed that each of these dressing materials have the ability to inhibit both *E. faecalis* and *P. gingivalis*.

The antibacterial properties of both root canal dressing materials mainly come from calcium hydroxide, which mechanism involve hydroxyl ion to kill bacteria by protein denaturation, 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 destruct lipid, the protein structure of bacteria and nucleic acid.[16] Direct contact of hydroxyl ion in alkaline pH with cytoplasmic membrane will destruct the hydrogen chain of protein polypeptide. Contact of hydroxyl ion with DNA will result in replication inhibition, causing lethal mutation. DNA is sensitive to temperature and pH change. In alkali condition DNA structure and function will break and leading to bacterial cell death. Alkali condition from calcium hydroxide would impact on the surrounding tissues, including healing, anti-inflammation[17] and cytotoxic which lead to apoptosis.[18,19]

The result of this study showed a significant difference between CH-Iodoform and CH-Barium Sulfate. The ability of CH-Iodoform to inhibit *E. faecalis* and *P. gingivalis* growths are significantly greater than CH-Barium Sulfate root canal dressing. In CH-Iodoform dressing, the iodoform substance will release iodine with high reactivity to promote protein oxidation. Iodoform functions as disinfectant and infection control. Iodoform and calcium hydroxide can diffuse into dentinal tubules for added disinfection and eventually will stimulate tissue repair.

In CH-Barium Sulfate, barium sulfate can diffuse into dentinal tubule and also perform antibacterial activity. In this study the antimicrobial strength is not as good as CH-Iodoform. This may be caused by the form and structure of barium sulfate used in the mixture. Previous study on antimicrobial of barium sulfate showed that barium sulfate in micron particulate form would have potent antimicrobial properties.[20] Barium sulfate is generally used for its radiopacity effect on radiographic examination.[11]

E. faecalis and *P. gingivalis* can be killed with high pH level. High pH or an extreme alkaline environment would disturb the survival of most bacteria.[21,22] However, there are some studies stated that pH of higher than 11.5 is required for potent disinfection.[23] This study showed inhibition zone of CH-Iodoform is two folds higher than CH-Barium Sulfate, this may be caused by the combination of these two materials, calcium hydroxide and iodoform would create synergistic effect on the antimicrobial activity to inhibit *E. faecalis* growth. Even though this dressing material won't eliminate *E. faecalis or P. gingivalis*, the material is expected to weaken the bacteria, and eventually body defense mechanism will be able eliminate these bacteria and their byproducts.

Many researches have been done to found novel, biocompatible, antimicrobial and regenerating root canal dressing materials in order to support successful root canal treatment and enhance patient's satisfaction. The success of root canal treatment aside from the right choice of dressing, there are other factors such as the root canal systems complexity, diffusion ability of dressing materials within the dentinal tubules, and the effect on mixed cultured bacteria or biofilm formation, and bacterial resistance, which need to be evaluated in further studies. As there are many microorganisms involved in failed root canal treatments, [24-26] even after biomechanical and chemical instrumentations during treatment, individual and personalized assessment of these bacteria or biofilm is needed for ideal treatment.

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In conclusion, calcium hydroxide-Iodoform root canal dressing has 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.

ACKNOWLEDGEMENT

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Dear Editor of DJMKG,

Thank you for giving me the opportunity to submit a revised draft of my manuscript titled **Antibacterial efficacy between calcium hydroxide-iodophors and calcium hydroxide-barium sulfate root canal dressings on *Enterococcus faecalis* and *Porphyromonas gingivalis in-vitro*** to Dental Journal (Majalah Kedokteran Gigi). We appreciate the time and effort that you and the reviewers have dedicated to providing your valuable feedback on our manuscript. We are grateful to the reviewers for their insightful comments on our manuscript. We have been able to incorporate changes to reflect most of the suggestions provided by the reviewers. We have highlighted the changes within the manuscript.

Here is a point-by-point response to the reviewers' comments and concerns.

No.	Reviewer comments	Before revision	Revision	Notes
Revision-1				
Reviewer 1 (RV1)				
1.	Reference number 23 to be replaced	Reference no 23	Replaced with reference number 6	Page: 6 Paragraph: 2
2.	Conclusion adjusted to the title	none	Added conclusion: calcium hydroxide-Iodoform root canal dressing has different (higher) antimicrobial efficacy on both *E. faecalis* and *P. gingivalis*.	Page: 6 Paragraph: 3
…				
Reviewer 2 (RV2)				
1.	Correction on page 2 paragraph 3	…resistance and periapical abnormalities…	…can spread to peri-radicular which causes the formation of peri-radicular lesions after root canal treatment.	Page: 2 Paragraph: 3
2.	Addition on page 2 paragraph 5	none	If done correctly, depending on the right instrumentation and irrigation to remove inorganic and organic smear layers.	Page: 2 Paragraph: 5
3.	Addition on page 3 paragraph 1	none	…patients who failed endodontic treatment	Page: 3 Paragraph: 1
4.	Explain about the modification	none	Modifications on the sum and position of the wells in the plates.	Page: 3 Paragraph: 2
5.	Correction on page 5 paragraph 2	There is no significance difference…. *E. faecalis* and *P. gingivalis*.	The sentences were removed for clarity	Page: 4 Paragraph: 2
6.	Addition on page 6 paragraph 5	none	Thus, the combination of CH and Iodoform will synergistically strengthen each other. … eliminate the bacteria.	Page: 5 Paragraph: 5

7.	Addition on page 7 paragraph 1	none	The addition of this material must consider its properties as it will affect the dressing's consistency and application.	Page: 6 Paragraph: 1
8.	Limitations of the study. Addition on page 7 paragraph 3	none	There are limitations, as this is only an in-vitro study and there are many factors to consider, both in-situ and in-vivo.	Page: 6 Paragraph: 3
…				
Managing Editor (ME)				
1.	Title correction	In-vitro antibacterial efficacy between calcium hydroxide-iodoform and calcium hydroxide-barium sulfate root canal dressings on *Enterococcus faecalis* and *Porphyromonas gingivalis*	Antibacterial efficacy between calcium hydroxide-iodoform and calcium hydroxide-barium sulfate root canal dressings on *Enterococcus faecalis* and *Porphyromonas gingivalis in-vitro*	Page: 1 Paragraph: title
2.	Purpose and conclusion	To determine…	To compare the antibacterial efficacy…	Page: 1 Paragraph: abstract
3.	Iodoform to be adjusted according to MeSH	iodoform	Iodophors (replaced all)	Page: all Paragraph: all
4.	Full and abbreviation of *E. faecalis* and *P. gingivalis*	none	*Enterococcus faecalis (E. faecalis* and *Porphyromonas gingivalis (P. gingivalis)*	Page: 2 Paragraph: 2
5.	Add the ethical clearance number	none	Ethical Clearance Commission (166/KKEPK.FKG)	Page: 3 Paragraph: 1
6.	Bacterial source	none	Stock bacteria of Research Center obtained from patients who failed endodontic treatment	
7.	Method modification	none	Modifications on the sum and position of the wells in the plates.	Page: 3 Paragraph: 2
8.	Sample size	none	According to methods used frequently in microbiology laboratory.	
9.	MIC and MBC	none	MIC and MBC were not conducted because this study use commercially available	

			products, not a basic substance or extracts.	
10.	Statistical analysis software	none	SPSS 20.0 for Windows (SPSS Inc., Chicago, Illinois, USA) was used in this study.	Page: 3 Paragraph: 6
11.	Tables and Figure	In the text	The tables and figure have been moved at the end, after the references	Page 10 and 11
12.	Limitation of the study	none	There are limitations, as this is only an in-vitro study and there are many factors to consider, both in-situ and in-vivo.	Page: 7 Paragraph: 3
...				
Revision-2				
Reviewer				
1.				Page: Paragraph:
2.				Page: Paragraph:
3.				Page: Paragraph:
4.				
5.				
...				

In addition to the above comments, all spelling and grammatical errors pointed out by the reviewers have been corrected. We look forward to hearing from you in due time regarding our submission and to respond to any further questions and comments you may have.
For improvement about the language editing, layout, tables and figure please arrange accordingly, thank you.

Sincerely,

Eric Priyo Prasetyo
Date: February 25, 2022.

Original article

Antibacterial efficacy between 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 which create an alkaline environment in the root canal and have 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 of CH-Iodophors and CH-Barium Sulfate on E. faecalis and P. gingivalis.* ***Methods:*** *CH-Iodophors and CH-Barium Sulfate was 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, 6 wells were used for CH-Iodophors group and 6 wells were used for CH-Barium Sulfate group. CH-Iodophors and CH-Barium Sulfate were deployed in the wells in E. faecalis and P. gingivalis cultured medium in the petri dishes. After incubation, the inhibition zone diameters were measured. Independent t-test was used for analysis and significance level was set at 5%.* ***Results****: There is a significant antibacterial efficacy between CH-Iodophors and CH-Barium Sulfate on E. faecalis and P. gingivalis (p=0.00001).* ***Conclusion:*** *CH-Iodophors has a higher antibacterial efficacy than CH-Barium Sulfate on both E. faecalis and P. gingivalis.*

Keywords: *Enterococcus faecalis; Porphyromonas gingivalis; calcium hydroxide; iodophors; 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 would result in patient's satisfaction. Many attempts were conducted to increase the success of root canal treatment, including finding the efficient instrumentation, effective cleaning,[1,2] antibacterial dressing and 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 are about 45.8-77% caused by *Enterococcus faecalis (E. faecalis)* and 28.17% caused by *Porphyromonas gingivalis (P. gingivalis)* in the root canal system.[6] Both microorganisms are among the ones that survive disinfecting protocol.[7]

E. faecalis can invade dentine tubules and can spread into peri-radicular which causes the formation of peri-radicular lesions after root canal treatment.[8] *P. gingivalis* can survive in the extra-radicular area mostly in the area approximate with root surface, and responsible for periodontitis and peri-radicular lesions.[9] *P. gingivalis* also contribute to root canal treatment failures, through its byproduct, lipopolysaccharide which effect biological processes, inflammation and tissue destruction.[10]

Different substances have been added to calcium hydroxide to increase its efficacy. The added substances are distilled water, saline, propylene glycol, chlorhexidine, glycerin, iodophors, barium sulfate, corticosteroids, antibiotics, anesthetic solution, methyl cellulose, and detergents. Iodophors has been added to calcium hydroxide to work with different bacterial characteristic.[11] Barium sulfate is added to calcium hydroxide aside from its antibacterial effect, it functions to increase radiopacity.[12]

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

MATERIALS AND METHODS

This is an experimental laboratory study, conducted in Conservative Dentistry Section, Universitas Airlangga Dental Hospital and Microbiology Laboratory, Research Center, Faculty of Dental Medicine, Universitas Airlangga. This study was ethically approved by 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 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 used in this study was agar diffusion method using Mueller Hinton (MH) agar and Brain Heart Infusion (BHI) broth. The method is according to Alharthi et al. (2021) and Balouiri et al. (2016) with modifications on the sum and position of the wells in the plates.[13,14] The media were allocated for 4 groups of experiments. In the first group, 12 wells were prepared for CH-Iodophors (6 wells with *E. faecalis* and 6 wells with *P. gingivalis*), and in the second group, 12 wells were prepared for CH-Barium Sulfate dressing (6 wells with *E. faecalis* and 6 wells with *P. gingivalis*).

Both *E. faecalis* and *P. gingivalis* bacterial culture from stock was moved into separate reaction tube, each containing BHI broth and stirred. Incubation was done for both culture at 37˚C for 24 hours in anaerobic condition. After 24 hours, *E. faecalis* and *P. gingivalis* bacterial culture in BHI broth was taken 0.5 ml using micro pipette, and poured into another reaction tube containing BHI broth until equal with 0.5 McFarland standard of turbidity.

The bacterial cultures were taken from BHI broth using sterile cotton swab and swabbed on the surface of each MH agar allocated for *E. faecalis* and *P gingivalis*. Antibacterial test was conducted by making the 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 millimeters) were collected for each sample wells.

Inhibition zones data were analyzed statistically and 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 Shapiro Wilk test. The significance was tested using independent t-test.

RESULTS

The number of replications (n) for each treatment group was 6. Mean and standard deviation of inhibition zone diameter of CH-Iodophors and CH-Barium Sulfate on *E. faecalis* and *P. gingivalis* are shown in Table 1. The mean of *E. faecalis* inhibition zone from CH-Iodophors was 11.8125 mm and CH-Barium Sulfate was 6.3750 mm. The mean of *P. gingivalis* inhibition zone from CH-Iodophors was 12.7875 mm and CH-Barium Sulfate was 6.6750 mm.

Independent t-test was used in this study to check the significance between CH-Iodophors group and CH-Barium Sulfate group on *E. faecalis* and *P. gingivalis*. The significance between inhibition zone diameter of CH-Iodophors and CH-Barium Sulfate on *E. faecalis* and *P. gingivalis* growth is shown in Table 2. We found a significant difference between the two treatment groups on both bacteria. The CH-Iodophors group has higher antibacterial efficacy with wider antibacterial clear zone in both bacteria ($p = 0.00001$). CH-Barium Sulfate group has lower antibacterial efficacy with narrower antibacterial clear zone in both bacteria.

DISCUSSION

Enterococcus faecalis and *Porphyromonas gingivalis* were used in this study because these bacteria are opportunistic, living in facultative anaerobic condition, found in pathogenic state, and associated with failed root canal treatment.[6] These bacteria also have a major role in persistent root canal infections, can survive in the root canals and resistant to commonly used intracanal dressings.[15]

Antibacterial efficacy of CH-Iodophors and CH-Barium Sulfate dressing materials on *E. faecalis* and *P. gingivalis* growths were experimentally checked with agar diffusion method and inhibition clear zone were measured on the media. Inhibition zone is a laboratory calculation method to check whether a material have the ability to inhibit bacterial growth.[14] The diameter of clear zones would describe the strength of such material to inhibit bacterial growth, the stronger this material inhibit, 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 drugs effect. Inhibition zones appeared on both groups of CH-Iodophors and CH-Barium Sulfate. This showed that each of these dressing materials have the ability to inhibit both *E. faecalis* and *P. gingivalis*.

The antibacterial properties of both root canal dressing materials mainly come from calcium hydroxide, which mechanism involve hydroxyl ion to kill bacteria by protein denaturation, cytoplasmic membrane and DNA destruction will physically inhibit this bacterial growth in the root canal systems.[16] Antibacterial activity is connected with alkali formation which can destruct lipid, the protein structure of bacteria and nucleic acid.[17] Direct contact of hydroxyl ion in alkaline pH with cytoplasmic membrane will destruct the hydrogen chain of protein polypeptide. Contact of hydroxyl ion with DNA will result in replication inhibition, causing lethal mutation. DNA is sensitive to temperature and pH change. In alkali condition DNA structure and function will break and leading to bacterial cell death. Alkali condition from calcium hydroxide would impact on the surrounding tissues, including healing, anti-inflammation[18] and cytotoxic which lead to apoptosis.[19,20]

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 are significantly greater than CH-Barium Sulfate root canal dressing. In CH-Iodophors dressing, the iodophors substance will release iodine with high reactivity to promote protein oxidation. Iodophors functions as disinfectant and infection control. Thus, the combination of

CH and Iodophors will synergistically strengthen each other. Iodophors and calcium hydroxide can diffuse into dentinal tubules for added disinfection and eliminate the bacteria.

In CH-Barium Sulfate, barium sulfate can diffuse into dentinal tubule and also perform antibacterial activity. In this study the antimicrobial strength is not as good as CH-Iodophors. This may be caused by the form and structure of barium sulfate used in the mixture. Previous study on antimicrobial of barium sulfate showed that barium sulfate in micron particulate form would have potent antimicrobial properties.[21] Barium sulfate is generally used for its radiopacity effect on radiographic examination.[12] The addition of this material must consider its properties as it will affect the dressing's consistency and application.

E. faecalis and *P. gingivalis* can be killed with high pH level. High pH or an extreme alkaline environment would disturb the survival of most bacteria.[22,23] However, there are some studies stated that pH of higher than 11.5 is required for potent disinfection.[6] This study showed inhibition zone of CH-Iodophors is two folds higher than CH-Barium Sulfate, this may be caused by the combination of these two materials, calcium hydroxide and iodophors would create synergistic effect on the antimicrobial activity to inhibit *E. faecalis* growth. Even though this dressing material won't eliminate *E. faecalis or P. gingivalis*, the material is expected to weaken the bacteria, and eventually body defense mechanism will be able eliminate these bacteria and their byproducts.

Many researches have been done to found novel, biocompatible, antimicrobial and regenerating root canal dressing materials in order to support successful root canal treatment and enhance patient's satisfaction. The success of root canal treatment aside from the right choice of dressing, there are other factors such as the root canal systems complexity, diffusion ability of dressing materials within the dentinal tubules, and the effect on mixed cultured bacteria or biofilm formation, and bacterial resistance, which need to be evaluated in further studies. As there are many microorganisms involved in failed root canal treatments, [24-26] even after biomechanical and chemical instrumentations during treatment, individual and personalized assessment of these bacteria or biofilm is 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, calcium hydroxide-Iodophors root canal dressing has 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.

ACKNOWLEDGEMENT

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Table 1:

Mean and standard deviation from inhibition zone diameter of CH-Iodophors and CH-Barium Sulfate on *E. faecalis* and *P. gingivalis*

Groups	**n**	***E. faecalis*** **Mean ± SD**	***P. gingivalis*** **Mean ± SD**
CH-Iodophors	6	11.8125 ± 1.32001	12.7875 ± 1.34961
CH-Barium Sulfate	6	6.3750 ± 0.19494	6.6750 ± 0.51865

n = replication

SD = standard deviation

Table 2: Significance between inhibition zone diameter of CH-Iodophors and CH-Barium Sulfate on *E. faecalis* and *P. gingivalis*

Groups	***E. faecalis*** **CH-Iodophors**	***P. gingivalis*** **CH-Iodophors**
E. faecalis **CH-Barium Sulfate**	0.00001*	-
P. gingivalis **CH-Barium Sulfate**	-	0.00001*

*Statistically significant

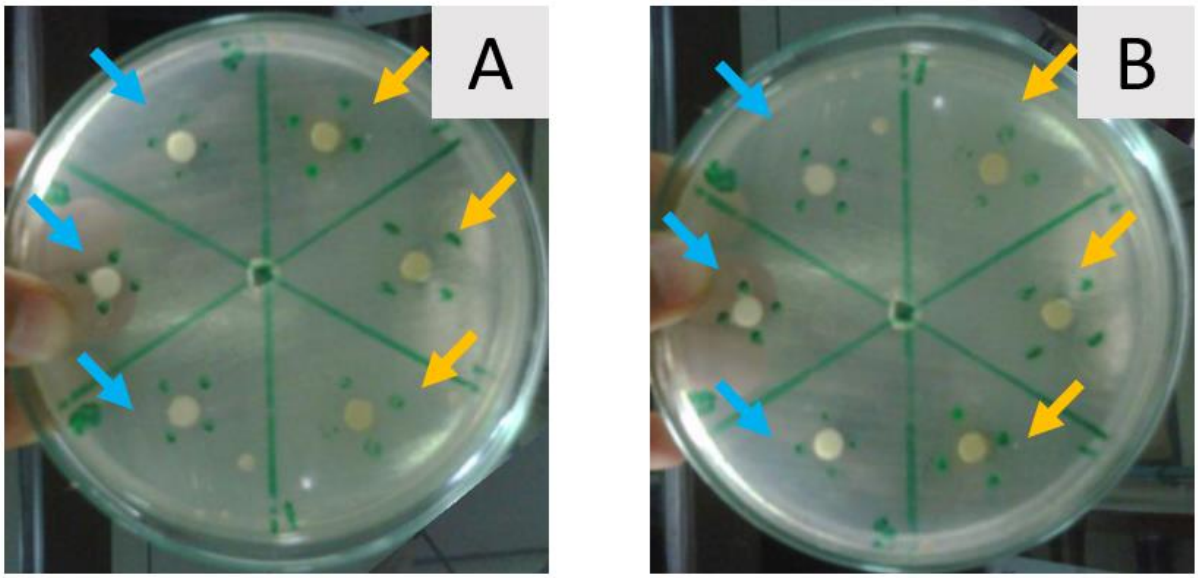

Figure 1.

Agar diffusion assay containing *E. faecalis* (A) and *P. gingivalis* (B). The green dots indicate the inhibition area of each well. CH-Iodophors is pointed by yellow arrows, and CH-Barium Sulfate is pointed by blue arrows.

eric priyo prasetyo <eric-p-p@fkg.unair.ac.id>

Editor decision: second revision required

3 messages

Dental Journal (Majalah Kedokteran Gigi) <dental_journal@fkg.unair.ac.id> Tue, Mar 8, 2022 at 10:44 AM
To: eric priyo prasetyo <eric-p-p@fkg.unair.ac.id>

Dear
Dr Eric

Please find the attached second review file from the reviewer.
Please revise according to the comments and highlight the text with color on the changes made.
Revisions, please send back no later than March 14, 2022 .
Thank you.

Regards,

Muhammad Dimas Aditya Ari

Dental Journal (Majalah Kedokteran Gigi)
http://e-journal.unair.ac.id/MKG

Faculty of Dental Medicine, Universitas Airlangga
Jl. Mayjen Prof. Dr. Moestopo 47 Surabaya 60132 INDONESIA
Phone/Fax: +62 31 5039478

review 2 Eric Priyo article_In-vitro antibacterial efficacy between calcium (BE).pdf
392K

eric priyo prasetyo <eric-p-p@fkg.unair.ac.id> Wed, Mar 9, 2022 at 1:07 AM
To: "Dental Journal (Majalah Kedokteran Gigi)" <dental_journal@fkg.unair.ac.id>

Dear Chief Editor,

Here is the 2nd revision and response letter. Please find them in the attachments.
Thank you.

Sincerely,
Dr. Eric & team.
[Quoted text hidden]

2 attachments

Response letter review Eric et al revision 2.pdf
181K

[DJMKG] Original article template CHI CHBS EF PG Eric et al Rev 2.docx
690K

Dental Journal (Majalah Kedokteran Gigi) <dental_journal@fkg.unair.ac.id> Wed, Mar 9, 2022 at 8:55 AM
To: eric priyo prasetyo <eric-p-p@fkg.unair.ac.id>

Dear
Drg Eric

Thank you for submitting a revised draft of your manuscript.
The manuscript will be re-evaluated by reviewers based on the revisions you make.

Best wishes,
Dental Journal (Majalah Kedokteran Gigi)
http://e-journal.unair.ac.id/MKG

Faculty of Dental Medicine, Universitas Airlangga
Jl. Mayjen Prof. Dr. Moestopo 47 Surabaya 60132 INDONESIA
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Original article

Antibacterial efficacy between calcium hydroxide-iodophors and calcium hydroxide-barium sulfate root canal dressings on *Enterococcus faecalis* and *Porphyromonas gingivalis in-vitro*

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 which create an alkaline environment in the root canal and have 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 of CH-Iodophors and CH-Barium Sulfate on E. faecalis and P. gingivalis.* ***Methods:*** *CH-Iodophors and CH-Barium Sulfate was 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, 6 wells were used for CH-Iodophors group and 6 wells were used for CH-Barium Sulfate group. CH-Iodophors and CH-Barium Sulfate were deployed in the wells in E. faecalis and P. gingivalis cultured medium in the petri dishes. After incubation, the inhibition zone diameters were measured. Independent t-test was used for analysis and significance level was set at 5%.* ***Results****: There is a significant antibacterial efficacy between CH-Iodophors and CH-Barium Sulfate on E. faecalis and P. gingivalis (p=0.00001).* ***Conclusion:*** *CH-Iodophors has a higher antibacterial efficacy than CH-Barium Sulfate on both E. faecalis and P. gingivalis.*

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

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Commented [BNR3]: managing editor's review has not been revised:
-compare the antibacterial efficacy between CH-Iodoform and CH-Barium Sulfate root canal dressing on E. faecalis and P. gingivalis.
-Please add the design and setting of this study
-How determine the isolated bacteria was E.faecalis or P.gingivalis?

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 would result in patient's satisfaction. Many attempts were conducted to increase the success of root canal treatment, including finding the efficient instrumentation, effective cleaning,[1,2] antibacterial dressing and 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 are about 45.8-77% caused by *Enterococcus faecalis (E. faecalis)* and 28.17% caused by *Porphyromonas gingivalis (P. gingivalis)* in the root canal system.[6] Both microorganisms are among the ones that survive disinfecting protocol.[7]

E. faecalis can invade dentine tubules and can spread into peri-radicular which causes the formation of peri-radicular lesions after root canal treatment.[8] *P. gingivalis* can survive in the extra-radicular area mostly in the area approximate with root surface, and responsible for periodontitis and peri-radicular lesions.[9] *P. gingivalis* also contribute to root canal treatment failures, through its byproduct, lipopolysaccharide which effect biological processes, inflammation and tissue destruction.[10]

Different substances have been added to calcium hydroxide to increase its efficacy. The added substances are distilled water, saline, propylene glycol, chlorhexidine, glycerin, iodophors, barium sulfate, corticosteroids, antibiotics, anesthetic solution, methyl cellulose, and detergents. Iodophors has been added to calcium hydroxide to work with different bacterial characteristic.[11] Barium sulfate is added to calcium hydroxide aside from its antibacterial effect, it functions to increase radiopacity.[12]

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

MATERIALS AND METHODS

This is an experimental laboratory study, conducted in Conservative Dentistry Section, Universitas Airlangga Dental Hospital and Microbiology Laboratory, Research Center, Faculty of Dental Medicine, Universitas Airlangga. This study was ethically approved by 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 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 used in this study was agar diffusion method using Mueller Hinton (MH) agar and Brain Heart Infusion (BHI) broth. The method is according to Alharthi et al. (2021) and Balouiri et al. (2016) with modifications on the sum and position of the wells in the plates.[13,14] The media were allocated for 4 groups of experiments. In the first group, 12 wells were prepared for CH-Iodophors (6 wells with *E. faecalis* and 6 wells with *P. gingivalis*), and in the second group, 12 wells were prepared for CH-Barium Sulfate dressing (6 wells with *E. faecalis* and 6 wells with *P. gingivalis*).

Both *E. faecalis* and *P. gingivalis* bacterial culture from stock was moved into separate reaction tube, each containing BHI broth and stirred. Incubation was done for both culture at 37˚C for 24 hours in anaerobic condition. After 24 hours, *E. faecalis* and *P. gingivalis* bacterial culture in BHI broth was taken 0.5 ml using micro pipette, and poured into another reaction tube containing BHI broth until equal with 0.5 McFarland standard of turbidity.

The bacterial cultures were taken from BHI broth using sterile cotton swab and swabbed on the surface of each MH agar allocated for *E. faecalis* and *P gingivalis.* Antibacterial test was conducted by making the 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 millimeters) were collected for each sample wells.

Inhibition zones data were analyzed statistically and 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 Shapiro Wilk test. The significance was tested using independent t-test.

RESULTS

The number of replications (n) for each treatment group was 6. Mean and standard deviation of inhibition zone diameter of CH-Iodophors and CH-Barium Sulfate on *E. faecalis* and *P. gingivalis* are shown in Table 1. The mean of *E. faecalis* inhibition zone from CH-Iodophors was 11.8125 mm and CH-Barium Sulfate was 6.3750 mm. The mean of *P. gingivalis* inhibition zone from CH-Iodophors was 12.7875 mm and CH-Barium Sulfate was 6.6750 mm.

Independent t-test was used in this study to check the significance between CH-Iodophors group and CH-Barium Sulfate group on *E. faecalis* and *P. gingivalis*. The significance between inhibition zone diameter of CH-Iodophors and CH-Barium Sulfate on *E. faecalis* and *P. gingivalis* growth is shown in Table 2. We found a significant difference between the two treatment groups on both bacteria. The CH-Iodophors group has higher antibacterial efficacy with wider antibacterial clear zone in both bacteria ($p = 0.00001$). CH-Barium Sulfate group has lower antibacterial efficacy with narrower antibacterial clear zone in both bacteria.

DISCUSSION

Enterococcus faecalis and *Porphyromonas gingivalis* were used in this study because these bacteria are opportunistic, living in facultative anaerobic condition, found in pathogenic state, and associated with failed root canal treatment.[6] These bacteria also have a major role in persistent root canal infections, can survive in the root canals and resistant to commonly used intracanal dressings.[15]

Antibacterial efficacy of CH-Iodophors and CH-Barium Sulfate dressing materials on *E. faecalis* and *P. gingivalis* growths were experimentally checked with agar diffusion method and inhibition clear zone were measured on the media. Inhibition zone is a laboratory calculation method to check whether a material have the ability to inhibit bacterial growth.[14] The diameter of clear zones would describe the strength of such material to inhibit bacterial growth, the stronger this material inhibit, 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 drugs effect. Inhibition zones appeared on both groups of CH-Iodophors and CH-Barium Sulfate. This showed that each of these dressing materials have the ability to inhibit both *E. faecalis* and *P. gingivalis*.

The antibacterial properties of both root canal dressing materials mainly come from calcium hydroxide, which mechanism involve hydroxyl ion to kill bacteria by protein denaturation, cytoplasmic membrane and DNA destruction will physically inhibit this bacterial growth in the root canal systems.[16] Antibacterial activity is connected with alkali formation which can destruct lipid, the protein structure of bacteria and nucleic acid.[17] Direct contact of hydroxyl ion in alkaline pH with cytoplasmic membrane will destruct the hydrogen chain of protein polypeptide. Contact of hydroxyl ion with DNA will result in replication inhibition, causing lethal mutation. DNA is sensitive to temperature and pH change. In alkali condition DNA structure and function will break and leading to bacterial cell death. Alkali condition from calcium hydroxide would impact on the surrounding tissues, including healing, anti-inflammation[18] and cytotoxic which lead to apoptosis.[19,20]

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 are significantly greater than CH-Barium Sulfate root canal dressing. In CH-Iodophors dressing, the iodophors substance will release iodine with high reactivity to promote protein oxidation. Iodophors functions as disinfectant and infection control. Thus, the combination of

CH and Iodophors will synergistically strengthen each other. Iodophors and calcium hydroxide can diffuse into dentinal tubules for added disinfection and eliminate the bacteria.

In CH-Barium Sulfate, barium sulfate can diffuse into dentinal tubule and also perform antibacterial activity. In this study the antimicrobial strength is not as good as CH-Iodophors. This may be caused by the form and structure of barium sulfate used in the mixture. Previous study on antimicrobial of barium sulfate showed that barium sulfate in micron particulate form would have potent antimicrobial properties.[21] Barium sulfate is generally used for its radiopacity effect on radiographic examination.[12] The addition of this material must consider its properties as it will affect the dressing's consistency and application.

E. faecalis and *P. gingivalis* can be killed with high pH level. High pH or an extreme alkaline environment would disturb the survival of most bacteria.[22,23] However, there are some studies stated that pH of higher than 11.5 is required for potent disinfection.[6] This study showed inhibition zone of CH-Iodophors is two folds higher than CH-Barium Sulfate, this may be caused by the combination of these two materials, calcium hydroxide and iodophors would create synergistic effect on the antimicrobial activity to inhibit *E. faecalis* growth. Even though this dressing material won't eliminate *E. faecalis or P. gingivalis*, the material is expected to weaken the bacteria, and eventually body defense mechanism will be able eliminate these bacteria and their byproducts.

Many researches have been done to found novel, biocompatible, antimicrobial and regenerating root canal dressing materials in order to support successful root canal treatment and enhance patient's satisfaction. The success of root canal treatment aside from the right choice of dressing, there are other factors such as the root canal systems complexity, diffusion ability of dressing materials within the dentinal tubules, and the effect on mixed cultured bacteria or biofilm formation, and bacterial resistance, which need to be evaluated in further studies. As there are many microorganisms involved in failed root canal treatments, [24-26] even after biomechanical and chemical instrumentations during treatment, individual and personalized assessment of these bacteria or biofilm is 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, calcium hydroxide-Iodophors root canal dressing has 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.

ACKNOWLEDGEMENT

The authors would like to thank the Publication Center, Dental Hospital, and Research Center, Faculty of Dental Medicine, Universitas Airlangga for the given facility.

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Table 1:

Mean and standard deviation from inhibition zone diameter of CH-Iodophors and CH-Barium Sulfate on *E. faecalis* and *P. gingivalis*

Groups	**n**	***E. faecalis*** **Mean ± SD**	***P. gingivalis*** **Mean ± SD**
CH-Iodophors	6	11.8125 ± 1.32001	12.7875 ± 1.34961
CH-Barium Sulfate	6	6.3750 ± 0.19494	6.6750 ± 0.51865

n = replication

SD = standard deviation

Table 2: Significance between inhibition zone diameter of CH-Iodophors and CH-Barium Sulfate on *E. faecalis* and *P. gingivalis*

Groups	***E. faecalis*** **CH-Iodophors**	***P. gingivalis*** **CH-Iodophors**
E. faecalis **CH-Barium Sulfate**	0.00001*	-
P. gingivalis **CH-Barium Sulfate**	-	0.00001*

*Statistically significant

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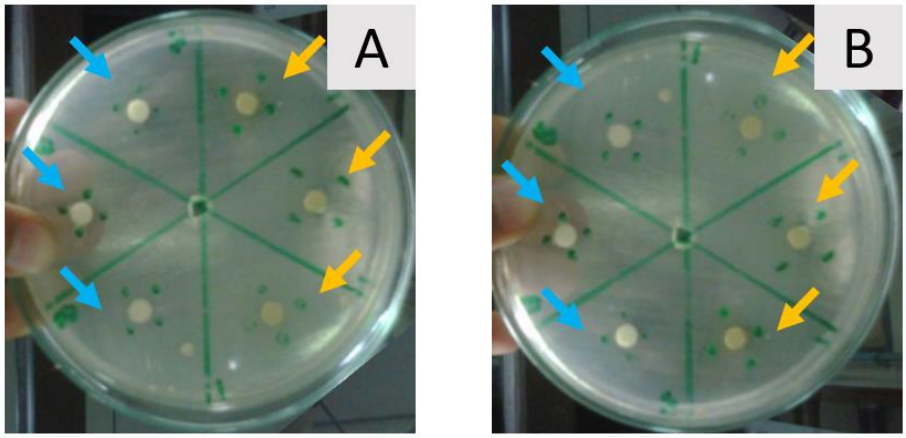

Figure 1.

Agar diffusion assay containing *E. faecalis* (A) and *P. gingivalis* (B). The green dots indicate the inhibition area of each well. CH-Iodophors is pointed by yellow arrows, and CH-Barium Sulfate is pointed by blue arrows.

Commented [BNR6]: managing editor's review has not been revised : The figure is not per instruction and not following the standard of the journal, please revise

Dear Editor of DJMKG,

Thank you for giving me the opportunity to submit a 2nd revision draft of my manuscript titled **Antibacterial efficacy between calcium hydroxide-iodophors and calcium hydroxide-barium sulfate root canal dressings on *Enterococcus faecalis* and *Porphyromonas gingivalis in-vitro*** to Dental Journal (Majalah Kedokteran Gigi). We appreciate the time and effort that you and the reviewers have dedicated to providing your valuable feedback on our manuscript. We are grateful to the reviewers for their insightful comments on our manuscript. We have been able to incorporate changes to reflect most of the suggestions provided by the reviewers. We have highlighted the changes within the manuscript.

Here is a point-by-point response to the reviewers' comments and concerns.

No.	Reviewer comments	Before revision	Revision	Notes
Revision-2				
Managing Editor (ME)				
1.	Managing editor's review has not been revised: why they add CH iodoform and Ch barium sulfate? This sentence unclear	Already explained, it is a commercially available medicament. Explanation is available in introduction and discussion.	Already explained, it is a commercially available medicament. Explanation is available in introduction and discussion.	Page: 1 Paragraph: Abstract
2.	A. Compare the antibacterial efficacy between CH-Iodoform and CH-Barium Sulfate root canal dressing on E. faecalis and P. gingivalis. B. Please add the design and setting of this study C. How determine the isolated bacteria was E. faecalis or P. gingivalis?	A. of …. B. This study is an in vitro. C. These bacteria are cultured and isolated by the microbiology laboratory of Research Center.	A. replaced B. Already available in the title, abstract and text, explained in detail in the materials and methods. C. Already available in the title, abstract and text, explained in detail in the materials and methods.	Page: 1 Paragraph: Abstract
3.	The old literature should be change	Reference no 9. Reference no 10, 11, 16.	Reference is removed. Reference is replaced.	Page: 7 Paragraph: References
4.	Please combine table 1 and table 2 into concise table	2 tables	1 table, combined.	Page: 10 Paragraph: Table
5.	The figure is not per instruction and not following the standard of the journal, please revise	The figure is missing in the text.	The figure is mentioned in the text (Results).	Page: 11 Paragraph: Figure
...				

In addition to the above comments, all spelling and grammatical errors pointed out by the reviewers have been corrected. We look forward to hearing from you in due time regarding our submission and to respond to any further questions and comments you may have.
For improvement about the language editing, layout, tables and figure please arrange accordingly, thank you.

Sincerely,

Eric Priyo Prasetyo
Date: March 8, 2022.

Original article

Antibacterial efficacy between calcium hydroxide-iodophors and calcium hydroxide-barium sulfate root canal dressings on *Enterococcus faecalis* and *Porphyromonas gingivalis in-vitro*

Eric Priyo Prasetyo,[1] Devi Eka Juniarti,[1] Galih Sampoerno,[1] Dian Agustin Wahjuningrum,[1] Ananta Tantri Budi,[1] Dyanita Hasri, [2] Evelyn Tjendronegoro[3]

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[3]Healthcare and Research, Irvine Medical Center, University of California, California, United States of America.

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 which create an alkaline environment in the root canal and have 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 dressing on E. faecalis and P. gingivalis.* ***Methods:*** *CH-Iodophors and CH-Barium Sulfate was 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, 6 wells were used for CH-Iodophors group and 6 wells were used for CH-Barium Sulfate group. CH-Iodophors and CH-Barium Sulfate were deployed in the wells in E. faecalis and P. gingivalis cultured medium in the petri dishes. After incubation, the inhibition zone diameters were measured. Independent t-test was used for analysis and significance level was set at 5%.* ***Results****: There is a significant antibacterial efficacy between CH-Iodophors and CH-Barium Sulfate on E. faecalis and P. gingivalis (p=0.00001).* ***Conclusion:*** *CH-Iodophors has a higher antibacterial efficacy than CH-Barium Sulfate on both E. faecalis and P. gingivalis.*

Keywords: *Enterococcus faecalis; Porphyromonas gingivalis; calcium hydroxide; iodophors; 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 would result in patient's satisfaction. Many attempts were conducted to increase the success of root canal treatment, including finding the efficient instrumentation, effective cleaning,[1,2] antibacterial dressing and 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 are about 45.8-77% caused by *Enterococcus faecalis (E. faecalis)* and 28.17% caused by *Porphyromonas gingivalis (P. gingivalis)* in the root canal system.[6] Both microorganisms are among the ones that survive disinfecting protocol.[7]

E. faecalis can invade dentine tubules and can spread into peri-radicular which causes the formation of peri-radicular lesions after root canal treatment.[8] *P. gingivalis* can survive in the extra-radicular area mostly in the area approximate with root surface, and responsible for periodontitis and peri-radicular lesions.[6] *P. gingivalis* also contribute to root canal treatment failures, through its byproduct, lipopolysaccharide which effect 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, glycerin, iodophors, barium sulfate, corticosteroids, antibiotics, anesthetic solution, methyl cellulose, and detergents. Iodophors has been added to calcium hydroxide to work with different bacterial characteristic.[10] Barium sulfate is added to calcium hydroxide aside from its antibacterial effect, it functions to increase radiopacity.[11]

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

MATERIALS AND METHODS

This is an experimental laboratory study, conducted in Conservative Dentistry Section, Universitas Airlangga Dental Hospital and Microbiology Laboratory, Research Center, Faculty of Dental Medicine, Universitas Airlangga. This study was ethically approved by 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 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 used in this study was agar diffusion method using Mueller Hinton (MH) agar and Brain Heart Infusion (BHI) broth. The method is according to Alharthi et al. (2021) and Balouiri et al. (2016) with modifications on the sum and position of the wells in the plates.[12,13] The media were allocated for 4 groups of experiments. In the first group, 12 wells were prepared for CH-Iodophors (6 wells with *E. faecalis* and 6 wells with *P. gingivalis*), and in the second group, 12 wells were prepared for CH-Barium Sulfate dressing (6 wells with *E. faecalis* and 6 wells with *P. gingivalis*).

Both *E. faecalis* and *P. gingivalis* bacterial culture from stock was moved into separate reaction tube, each containing BHI broth and stirred. Incubation was done for both culture at 37˚C for 24 hours in anaerobic condition. After 24 hours, *E. faecalis* and *P. gingivalis* bacterial culture in BHI broth was taken 0.5 ml using micro pipette, and poured into another reaction tube containing BHI broth until equal with 0.5 McFarland standard of turbidity.

The bacterial cultures were taken from BHI broth using sterile cotton swab and swabbed on the surface of each MH agar allocated for *E. faecalis* and *P gingivalis*. Antibacterial test was conducted by making the 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 millimeters) were collected for each sample wells.

Inhibition zones data were analyzed statistically and 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 Shapiro Wilk test. The significance was tested using independent t-test.

RESULTS

The number of replications (n) for each treatment group was 6. Mean and standard deviation of inhibition zone diameter of CH-Iodophors and CH-Barium Sulfate on *E. faecalis* and *P. gingivalis* are shown in Table 1. The mean of *E. faecalis* inhibition zone from CH-Iodophors was 11.8125 mm and CH-Barium Sulfate was 6.3750 mm. The mean of *P. gingivalis* inhibition zone from CH-Iodophors was 12.7875 mm and CH-Barium Sulfate was 6.6750 mm.

Independent t-test was used in this study to check the significance between CH-Iodophors group and CH-Barium Sulfate group on *E. faecalis* and *P. gingivalis*. The significance between inhibition zone diameter of CH-Iodophors and 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 wider antibacterial clear zone in both bacteria ($p = 0.00001$). CH-Barium Sulfate group has lower antibacterial efficacy with narrower antibacterial clear zone in both bacteria. 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 pathogenic state, and associated with failed root canal treatment.[6] These bacteria also have a major role in persistent root canal infections, can survive in the root canals and resistant to commonly used intracanal dressings.[14]

Antibacterial efficacy of CH-Iodophors and CH-Barium Sulfate dressing materials on *E. faecalis* and *P. gingivalis* growths were experimentally checked with agar diffusion method and inhibition clear zone were measured on the media. Inhibition zone is a laboratory calculation method to check whether a material have the ability to inhibit bacterial growth.[13] The diameter of clear zones would describe the strength of such material to inhibit bacterial growth, the stronger this material inhibit, 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 drugs effect. Inhibition zones appeared on both groups of CH-Iodophors and CH-Barium Sulfate. This showed that each of these dressing materials have the ability to inhibit both *E. faecalis* and *P. gingivalis*.

The antibacterial properties of both root canal dressing materials mainly come from calcium hydroxide, which mechanism involve hydroxyl ion to kill bacteria by protein denaturation, 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 destruct lipid, the protein structure of bacteria and nucleic acid.[16] Direct contact of hydroxyl ion in alkaline pH with cytoplasmic membrane will destruct the hydrogen chain of protein polypeptide. Contact of hydroxyl ion with DNA will result in replication inhibition, causing lethal mutation. DNA is sensitive to temperature and pH change. In alkali condition DNA structure and function will break and leading to bacterial cell death. Alkali condition from calcium hydroxide would impact on the surrounding tissues, including healing, anti-inflammation[17] and cytotoxic which lead 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 are significantly greater than CH-Barium Sulfate root canal dressing. In CH-Iodophors dressing, the iodophors substance will release iodine with high reactivity to promote protein oxidation. Iodophors functions as disinfectant and infection control. Thus, the combination of

CH and Iodophors will synergistically strengthen each other. Iodophors and calcium hydroxide can diffuse into dentinal tubules for added disinfection and eliminate the bacteria.

In CH-Barium Sulfate, barium sulfate can diffuse into dentinal tubule and also perform antibacterial activity. In this study the antimicrobial strength is not as good as CH-Iodophors. This may be caused by the form and structure of barium sulfate used in the mixture. Previous study on antimicrobial of barium sulfate showed that barium sulfate in micron particulate form would have potent antimicrobial properties.[20] Barium sulfate is generally used for its radiopacity effect on radiographic examination.[11] The addition of this material must consider its properties as it will affect the dressing's consistency and application.

E. faecalis and *P. gingivalis* can be killed with high pH level. High pH or an extreme alkaline environment would disturb the survival of most bacteria.[21,22] However, there are some studies stated that pH of higher than 11.5 is required for potent disinfection.[6] This study showed inhibition zone of CH-Iodophors is two folds higher than CH-Barium Sulfate, this may be caused by the combination of these two materials, calcium hydroxide and iodophors would create synergistic effect on the antimicrobial activity to inhibit *E. faecalis* growth. Even though this dressing material won't eliminate *E. faecalis or P. gingivalis*, the material is expected to weaken the bacteria, and eventually body defense mechanism will be able eliminate these bacteria and their byproducts.

Many researches have been done to found novel, biocompatible, antimicrobial and regenerating root canal dressing materials in order to support successful root canal treatment and enhance patient's satisfaction. The success of root canal treatment aside from the right choice of dressing, there are other factors such as the root canal systems complexity, diffusion ability of dressing materials within the dentinal tubules, and the effect on mixed cultured bacteria or biofilm formation, and bacterial resistance, 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 personalized assessment of these bacteria or biofilm is 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, calcium hydroxide-Iodophors root canal dressing has 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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Table 1:

Mean, standard deviation, and significance from inhibition zone diameter of CH-Iodophors and CH-Barium Sulfate on *E. faecalis* and *P. gingivalis*

Groups	**n**	***E. faecalis*** **Mean ± SD**	**P value**	***P. gingivalis*** **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	

n = replication

SD = standard deviation

*= statistically significant

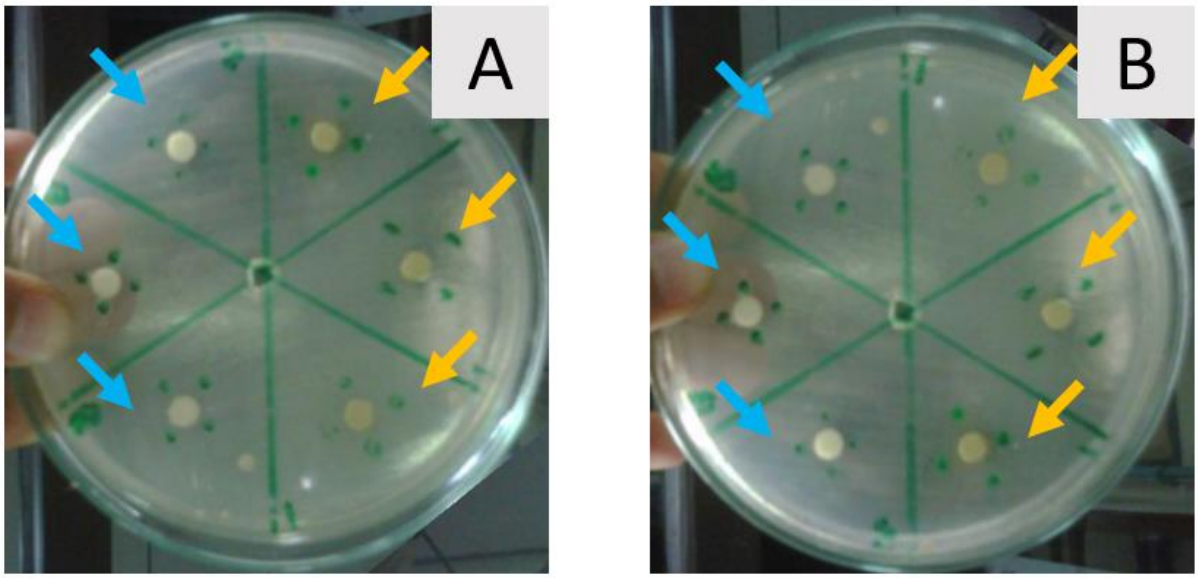

Figure 1.

Agar diffusion assay containing *E. faecalis* (A) and *P. gingivalis* (B). The green dots indicate the inhibition area of each well. CH-Iodophors is pointed by yellow arrows, and CH-Barium Sulfate is pointed by blue arrows.

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Acceptance letter

Dental Journal (Majalah Kedokteran Gigi) <dental_journal@fkg.unair.ac.id> Wed, Mar 16, 2022 at 9:30 AM
To: eric priyo prasetyo <eric-p-p@fkg.unair.ac.id>, Eric Prasetyo <eppfkgunair@gmail.com>

Dear

Eric Priyo Prasetyo,
Faculty of Dental Medicine, Universitas Airlangga
Surabaya, Indonesia

We have reached a decision regarding your submission to Dental Journal (Majalah Kedokteran Gigi),
Effects of tooth preparation on the microleakage of fissure sealantAntibacterial efficacy between calcium hydroxide-iodophors and calcium hydroxide-barium sulfate root canal dressings on Enterococcus faecalis and Porphyromonas gingivalis in-vitro.
Authors: Eric Priyo Prasetyo, Devi Eka Juniarti, Galih Sampoerno, Dian Agustin Wahjuningrum, Ananta Tantri Budi, Dyanita Hasri, Evelyn Tjendronegoro
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