



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

Manuscript submitted to Dove Medical Press

1 message

Mr Chesnokov <borischesnokov@dovepress.com>
 Reply-To: Mr Chesnokov <borischesnokov@dovepress.com>
 To: Dr Prasetyo <eric-p-p@fkg.unair.ac.id>

Sun, Sep 27, 2020 at 11:25 PM

Dear Dr Prasetyo,

Thank you for your recent submission to Clinical, Cosmetic and Investigational Dentistry, titled "Calcium Hydroxide Increases Human Umbilical Cord Mesenchymal Stem Cells Expressions of Apoptotic Protease-Activating Factor-1, Caspase-3 and Caspase-9" which has been received.

You uploaded the following files with this submission:

284240-ms.doc

284240-turnitin-16--calcium-hydroxide-increases-human-umbilical-cord-mesenchymal-stem-cells-expressions-of-apoptotic-protease-activating-factor-1-caspase-3-and-caspase-9-2-.pdf

CONFLICT OF INTEREST DISCLOSURE

Please now complete the disclosure form by clicking on the following link:

https://www.dovepress.com/icmje_coi.php?submission_id=284240&author_id=1378802&I=hKjiDbgWVCMkj2TVT85EIScy1378802

The purpose of this form is to provide the Editor-in-Chief of Clinical, Cosmetic and Investigational Dentistry with important information about your possible conflicts of interests. The composition of this form follows that of the International Committee of Medical Journal Editors (ICMJE) standard and further demonstrates our commitment to the highest ethical and professional standards.

The form is designed to be completed and stored electronically. Each author will receive an individual email like this and should submit a separate form. Each author is responsible for the accuracy and completeness of the submitted information.

What happens next:

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Your submission will be given an initial review to ensure its suitability for Clinical, Cosmetic and Investigational Dentistry. Once that has been completed, peer review will commence, and we will be in contact again when that has been completed.

If your paper is accepted for publication you will need to pay an article publishing charge of USD 1958 (plus VAT if applicable).

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At any time throughout the submission process you are welcome to contact the Editorial Team should you have any questions about your submission. The status of your submission can also be tracked through DoveCentral. You will automatically be notified of changes in the status of your submission.

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Please note that your submission has been received on the basis of your agreement with the terms and conditions which you were asked to consent to during the submission process. These are outlined again below and are available in full on the website (http://www.dovepress.com/author_guidelines.php?content_id=771):

- The submission is in compliance with the author guidelines and any applicable journal-specific guidelines; and,
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- I have read and understood the copyright terms; and,
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- The manuscript I am submitting is not currently under consideration for publication in another journal, nor has it been published in another journal; and,
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- I absolve Dove Medical Press Ltd from all legal liability arising from my submission; and,
- I have taken due care to ensure integrity of the submission and according to currently accepted scientific knowledge all statements in it purporting to be facts are true.

Some institutions have an open access fund available to their researchers, which can help to pay for the publication processing fee. We encourage you to contact your institution library to enquire if this is available to you.

Changes to authorship

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Dove does not permit the changing/adding/deleting of authors after submission of the paper. We support the GPP3 guidelines that indicate addition or removal of an author should only happen in rare cases, such as the work changing substantially in response to the reviewer or Editor's comments.

Many thanks for your submission.

Yours sincerely

Mr Chesnokov
Editorial Department
Dove Medical Press
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(ID: 284240)



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

Manuscript Update Clinical, Cosmetic and Investigational Dentistry [Sub ID 284240]

1 message

Mrs Finn <beverleyfinn@dovepress.com>
Reply-To: Mrs Finn <beverleyfinn@dovepress.com>
To: Dr Prasetyo <eric-p-p@fkg.unair.ac.id>

Tue, Sep 29, 2020 at 6:53 PM

Dear Dr Prasetyo

Your manuscript Calcium Hydroxide Increases Human Umbilical Cord Mesenchymal Stem Cells Expressions of Apoptotic Protease-Activating Factor-1, Caspase-3 and Caspase-9 submitted to Clinical, Cosmetic and Investigational Dentistry has been sent for peer review.

Most of the submission process is automated and you can follow the progress of your manuscript by logging into your Dove account. For an explanation of the different status indicators please click on the following link:
https://www.dovepress.com/author_guidelines.php?content_id=2126

Our publication policy specifies that every manuscript must have a minimum of two sets of peer review comments in order that the editorial decision-maker can reach a conclusion on the manuscript. When we have the required number of peer review reports returned your manuscript will be sent for first editorial decision. Once we have been advised of the Editor-in-Chief's decision you will be notified by email and provided with the peer-reviewers comments.

If I can be of further assistance, please do not hesitate to contact me.

Kind regards
Mrs Finn
Clinical, Cosmetic and Investigational Dentistry
Dove Medical Press



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

Your manuscript has been sent to the Editor-in-Chief [ID 284240]

1 message

Ms Lawrence <shanilawrence@dovepress.com>
Reply-To: Ms Lawrence <shanilawrence@dovepress.com>
To: eric-p-p@fkg.unair.ac.id

Tue, Nov 10, 2020 at 2:58 AM

Dear Dr Prasetyo

Journal Name: Clinical, Cosmetic and Investigational Dentistry
Title: Calcium Hydroxide Increases Human Umbilical Cord Mesenchymal Stem Cells Expressions of Apoptotic Protease-Activating Factor-1, Caspase-3 and Caspase-9
ID: 284240
Author: Dr Prasetyo

We are happy to advise that your manuscript and comments from the peer-reviewers have been delivered to our Editor-in-Chief to review and comment. We will be in touch shortly with the outcome.

Please note that the decision of the Editor-in-Chief whether to accept or reject any paper is full and final.

Please do not hesitate to contact us if you have any questions.

Don't forget to register for email alerts to this journal by clicking here:

https://www.dovepress.com/quick_signup.php?journal_id=54&l=hKjiDbgWVCMkj2TVT85EIScy1378802

Sincerely,

Ms Lawrence
Editorial Department
Dove Medical Press Ltd
Live Chat: http://www.dovepress.com/live_help.t
Twitter: <https://twitter.com/DovePress>
Facebook: <https://www.facebook.com/DoveMedicalPress>
www.dovepress.com - open access to scientific and medical research



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

Manuscript submitted to Dove Medical Press - Response Required

2 messages

Sonam Patel <kajalpatel@dovepress.com>
Reply-To: Sonam Patel <kajalpatel@dovepress.com>
To: Dr Prasetyo <eric-p-p@fkg.unair.ac.id>

Tue, Nov 17, 2020 at 8:42 AM

Dear Dr Prasetyo,

Thank you for your manuscript submission to Clinical, Cosmetic and Investigational Dentistry. On behalf of the Editor, I would like to inform you that your submitted manuscript 'Calcium Hydroxide Increases Human Umbilical Cord Mesenchymal Stem Cells Expressions of Apoptotic Protease-Activating Factor-1, Caspase-3 and Caspase-9' (284240) has been peer-reviewed and may be considered for publication after the necessary revisions are completed to the Editors satisfaction.

IMPORTANT

We require you to confirm that you wish to proceed and intend to submit a revised manuscript within 21 days. You can do this by confirming your intention to revise by using the calendar supplied on your author dashboard; or you can reply to this email.

https://www.dovepress.com/manuscript_revision.php?submission_id=284240&l=hKjiDbgWVCMkj2TVT85EIScy1378802

Once you confirm your intention to revise, we will send a confirmation email which contains a link to submit your revised files.

If, after you have considered the reviewer comments, you decide that you require longer than 21 days to revise and resubmit, please let us know immediately.

EDITOR EVALUATION

You can download your reviewer comments from your author interface below:

https://www.dovepress.com/manuscript_revision.php?pa=reviewer_comment&submission_id=284240&l=hKjiDbgWVCMkj2TVT85EIScy1378802

ETHICAL CORRECTIONS.

- Ethics. Please be advised that we are aware of any current ethics statements and are requesting further detail. The manuscript cannot proceed past the editorial stage without addressing the following. Please add the updated statement to both your manuscript and response letter:

HUCMSCs: Please confirm in the revised manuscript that the cell donors provided informed consent, in accordance with the Declaration of Helsinki.

If you have any queries regarding the ethic requirements, please view our Frequently Asked Questions:

https://www.dovepress.com/cr_data/ethics-faq-2019.pdf

EDITORIAL CORRECTIONS:

- Figure File Type: Please supply all figures in high quality .jpg, .tif or .pdf format, one file for each figure (line art 900 dpi, combination [line art + halftone] 900 dpi, halftone 300 dpi). If the figures have also been placed in your manuscript, please remove.

- Please place the figure legends at the end of the manuscript or in a separate word document.

- Figure Presentation: All current figures feature spelling and grammar issues, please carefully check all spelling and grammar prior to re-submission as the figures supplied this stage will be the ones sent for publication. Please carefully revise your figures to ensure they follow our Figure Guidelines regarding accepted fonts, line type, and image sizing.

- Please ensure copies of all figures/tables/supplementary material are provided with the revised manuscript, even if these are not altered during the revisions so we can ensure we have the most up to date file for each.

When submitting your revised manuscript, please also provide a separate response letter addressed to the Editor. Please address every comment made by the Reviewers and Editor, and all the requested Editorial and Ethical corrections in both the manuscript and response letter. This will ensure your revised manuscript proceeds through our system without delays. Any comments or corrections not addressed or responded to will result in your submission being placed on hold while we await the corrections to be made.

Kind Regards,

Sonam Patel
On behalf of Professor Christopher E. Okunseri
Editorial Department
Dove Medical Press
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eric priyo prasetyo <eric-p-p@fkg.unair.ac.id>
To: Sonam Patel <kajalpatel@dovepress.com>

Fri, Nov 20, 2020 at 4:20 PM

Dear Sonam Patel,

Thank you for your email. Yes, we'd like to proceed.
We'll do our best to adjust with the corrections and submit a revised manuscript within 21 days.
Thank you once again.

Best regards,
Eric & Team.
[Quoted text hidden]



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

Dove Medical Press – Confirmation of Revision Period

1 message

Sonam Patel <kajalpatel@dovepress.com>
Reply-To: Sonam Patel <kajalpatel@dovepress.com>
To: Dr Prasetyo <eric-p-p@fkg.unair.ac.id>

Mon, Nov 23, 2020 at 1:38 AM

Dear Dr Prasetyo

Titled: Calcium Hydroxide Increases Human Umbilical Cord Mesenchymal Stem Cells Expressions of Apoptotic Protease-Activating Factor-1, Caspase-3 and Caspase-9
Submission ID: 284240

Thank you for confirming your intention to submit a revised manuscript for Clinical, Cosmetic and Investigational Dentistry. I have noted a due date of 14 Dec 2020 in our system. If you require further time, please let us know as soon as possible. We look forward to receiving your revised manuscript.

When submitting your revised manuscript, please include a point-by-point response letter. This letter must contain all comments made by the reviewers, the Editor and the editorial staff, and your responses to these comments. The response letter assists the Editor in their final decision.

Dove Medical Press does not generally permit changes to the author list of a submitted manuscript. As per our Authorship Policies (https://www.dovepress.com/editorial_policies.php?content_id=3521) we support the GPP3 guidelines which indicate that the addition or removal of an author should only occur in rare cases, such as the work undergoing substantial revisions in response to reviewer or Editor comments.

Please use the following link to submit your revised manuscript, response letter and any additional revised or requested files.

https://www.dovepress.com/upload_files_new.php?submission_id=284240&l=hKjiDbgWVCMkj2TVT85EIScy1378802

VIDEO ABSTRACT

We would also like to invite you to submit a short video abstract, which will be published with your paper. This is an initiative that encourages videos to be presented by the author(s). The video should be of 1-3 minutes duration and give an overview of their paper, so readers can get an idea of the content and motivation behind the paper.

If you are able to prepare a video abstract for this paper please ensure this is completed and submitted at the same time as your revised manuscript, as we will be unable to accept a video abstract at a later stage in the process. In the meantime the guidelines and an example video abstract are available here:

https://www.dovepress.com/author_guidelines.php?content_id=3195

Kind regards

Sonam Patel
kajalpatel@dovepress.com
Dove Medical Press
[ID 284240]

Manuscript ID number:

284240

Title of paper:

Calcium Hydroxide Increases Human Umbilical Cord Mesenchymal Stem Cells Expressions of Apoptotic Protease-Activating Factor-1, Caspase-3 and Caspase-9

Reviewer 1

Evaluations (peer review comments for the author)

1. In general, how do you rate the degree to which the paper is easy to follow and its logical flow?

Good

2. Do the title and abstract cover the main aspects of the work?

No. The abstract is truncated and some important information is missing.

3. If relevant are the results novel? Does the study provide an advance in the field?

Yes

4. Did the study gain ethical approval appropriate to the country in which the research was performed if human or animal subjects, human cell lines or human tissues were involved and is it stated in the manuscript?

Yes

Does the paper raise any ethical concerns?

No

5. If relevant, are the methods clear and replicable?

Yes

6. If relevant, do all the results presented match the methods described?

Yes

7. If relevant, is the statistical analysis appropriate to the research question and study design?

Yes

8. If relevant, is the selection of the controls appropriate for the study design. Have attempts been made to address potential bias through analytic methods, eg., sensitivity analysis

NA

9. How do you rate how clearly and appropriately the data are presented

Good

10. If relevant, did the authors, make the underlying data available to the readers?

Yes

11. Do the conclusions correlate to the results found?

Yes

12. Are the figures and tables clear and legible?

Yes

Are images clear and free from unnecessary modification?

Yes

13. I have serious concerns about the validity of this manuscript

No

14. Does the paper use appropriate references in the correct style to promote understanding of the content?

Yes

15. If relevant, do any of the authors competing interests raise concerns about the validity of the study i.e. have the authors' competing interests created a bias in the reporting of the results and conclusions?.

No

16. Do you think the manuscript requires English editing to correct the grammar or flow?

No

Evaluation

I would like to congratulate the authors for the article, it is possible to see that a lot of effort was made to conduct this study. In general, the study is interesting, current and brings new perspectives and future directions for new research in the area of regenerative endodontics. I have only small comments about the manuscript:

ABSTRACT:

Purpose: The text is all truncated, with small sentences.

Material and Methods: I would like the authors to add which technique is used to evaluate APAF-1, Caspase-3, and Caspase-9 biomarkers.

Results: The authors described that "The addition of calcium hydroxide in MEM Alpha medium increases HUCMSCs expression of APAF-1, caspase-3, and caspase-9 significantly, compared to the control group without calcium hydroxide (p <0.05)" - in all the times? Please make this clearer.

INTRODUCTION:

The authors should clarify the importance of specifically evaluating these biomarkers and their relationship to stem cell survival. I also suggest that the authors define the hypothesis that will be tested, right after the objectives.

DISCUSSION:

The authors conducted a very detailed discussion of the findings obtained. However, I would like the authors to reflect on how these results may imply clinical practice during regenerative endodontic procedures. Also, as an in vitro study, they would like the authors to point out the limitations existing within the methodology and directions for future studies

Reviewer 2

Evaluations (peer review comments for the author)

1. In general, how do you rate the degree to which the paper is easy to follow and its logical flow?

Good

2. Do the title and abstract cover the main aspects of the work?

Yes

3. If relevant are the results novel? Does the study provide an advance in the field?

Yes

4. Did the study gain ethical approval appropriate to the country in which the research was performed if human or animal subjects, human cell lines or human tissues were involved and is it stated in the manuscript?

Yes

Does the paper raise any ethical concerns?

No

5. If relevant, are the methods clear and replicable?

Yes. Generally, methods section must be cited with related references.

6. If relevant, do all the results presented match the methods described?

Yes

7. If relevant, is the statistical analysis appropriate to the research question and study design?

Yes

8. If relevant, is the selection of the controls appropriate for the study design. Have attempts been made to address potential bias through analytic methods, eg., sensitivity analysis

Yes

9. How do you rate how clearly and appropriately the data are presented

Good

10. If relevant, did the authors, make the underlying data available to the readers?

Yes

11. Do the conclusions correlate to the results found?

Yes

12. Are the figures and tables clear and legible?

Yes

Are images clear and free from unnecessary modification?

Yes

13. I have serious concerns about the validity of this manuscript

No

14. Does the paper use appropriate references in the correct style to promote understanding of the content?

No. Generally, methods section must be cited with related references.

15. If relevant, do any of the authors competing interests raise concerns about the validity of the study i.e. have the authors' competing interests created a bias in the reporting of the results and conclusions?.

No

16. Do you think the manuscript requires English editing to correct the grammar or flow?

No

Evaluation

The results presented in the manuscript entitled "Calcium Hydroxide Increases Human Umbilical Cord Mesenchymal Stem Cells Expressions of Apoptotic Protease-Activating Factor-1, Caspase-3 and Caspase-9" are interesting to warrant publication in "Clinical, Cosmetic and Investigational Dentistry" after major revision.

1)The introduction section must be improved and at the end of the introduction section, please mention the hypothesis and novelty of your study. You can use and refer the following paper regarding the role and important of mesenchymal stem cells in cell-based therapy:

□Stem cell-based regenerative medicine. Stem Cell Investigation. 2019;6:19. Doi:10.21037/sci.2019.06.04

2)Which passage of cells were used? Please specify in the text of manuscript.

3)Generally, methods section must be cited with related references.

4)The identification and characterization method of isolated mesenchymal stem cells must be discussed in detail. This comment is required. You can use and refer the following papers which explained elaborately and completely the "flow cytometric analysis and multi-lineage differentiation method for characterization of mesenchymal stem cells" in "Preparation of HUCMSCs and Calcium Hydroxide" section:

□Interleukin-6, -8, and TGF- β Secreted from Mesenchymal Stem Cells Show Functional Role in Reduction of Telomerase Activity of Leukemia Cell Via Wnt5a/ β -Catenin and P53 Pathways.

□L-carnitine significantly decreased aging of rat adipose tissue-derived mesenchymal stem cells. Veterinary research communications. 2017 Mar 1;41(1):41-7.

5)Also, the immunocytochemistry method must be explained in detail. You can use and refer the following paper which explained and analyzed elaborately the "immunocytochemistry method" in "Evaluation of apoptosis" section:

□Mesenchymal Stem Cells Could Be Considered as a Candidate for Further Studies in Cell-Based Therapy of Alzheimer's Disease via Targeting the Signaling Pathways. ACS Chemical Neuroscience. 2020 Apr 20;11(10):1424-35.

6)Also, since the discussion section is one of the most important parts of the paper, this section must be improved with more attention and explanation. In the discussion section, results must be compared with another results from previous studies.

To:
The Editor
Clinical, Cosmetic and Investigational Dentistry

Surabaya, 8 December 2020

Dear Editor,

Thank you very much for the corrections to better improve our manuscript. Hereby we provide a response letter addressed to you regarding the corrections from ethical, editorial, and reviewers for:

Manuscript ID number:

284240

Title of paper:

Calcium Hydroxide Increases Human Umbilical Cord Mesenchymal Stem Cells Expressions of Apoptotic Protease-Activating Factor-1, Caspase-3 and Caspase-9

We have made corrections as suggested by the ethical, editorial, and reviewers (provided in yellow highlights in the manuscript) as follows:

Ethical Corrections:

Response: Corrections have been made, the statement of the cell donors provided informed consent, in accordance with the Declaration of Helsinki has been added to the manuscript on page 4 line 22.

Editorial Corrections:

Response: Figure file has been supplied in a separate tiff format. Figures placed in the manuscript have been removed. Figure presentation has been checked and up to date.

Figure and Table legends have been placed at the end of the manuscript (after references) and in a separate word document.

Response to Reviewer 1:

Dear Reviewer 1, thank you very much for the review, we hope that our response would be sufficient and acceptable for you. Revisions were conducted and marked in yellow highlights.

ABSTRACT:

Purpose: The text is all truncated, with small sentences.

Response: We have added and lengthen some sentences on page 2 line 11 and 12.

Material and Methods: I would like the authors to add which technique is used to evaluate APAF-1, Caspase-3, and Caspase-9 biomarkers.

Response: We have added the technique on page 2 line 19, 20, and 21.

Results: The authors described that "The addition of calcium hydroxide in MEM Alpha medium increases HUCMSCs expression of APAF-1, caspase-3, and caspase-9 significantly, compared to the control group without calcium hydroxide ($p < 0.05$)" - in all the times? Please make this clearer.

Response: We have added the sentence to better clear the result on page 3 line 5.

INTRODUCTION:

The authors should clarify the importance of specifically evaluating these biomarkers and their relationship to stem cell survival. I also suggest that the authors define the hypothesis that will be tested, right after the objectives.

Response: We have added the importance of evaluating these markers and their relationship to HUCMSCs' survival on page 4 line 7-10. We have added the hypothesis on page 4 line 13-15.

DISCUSSION:

The authors conducted a very detailed discussion of the findings obtained. However, I would like the authors to reflect on how these results may imply clinical practice during regenerative endodontic procedures. Also, as an in vitro study, they would like the authors to point out the limitations existing within the methodology and directions for future studies

Response: We have added the implication on regenerative endodontic procedures and the limitations of this study on page 10 line 15-20.

Response to Reviewer 2:

Dear Reviewer 2, thank you for the supportive review to better improve our manuscript, we hope that our response would be sufficient and acceptable for you. Revisions were conducted and marked in yellow highlights.

1)The introduction section must be improved and at the end of the introduction section, please mention the hypothesis and novelty of your study. You can use and refer the following paper regarding the role and important of mesenchymal stem cells in cell-based therapy:

• Stem cell-based regenerative medicine. Stem Cell Investigation. 2019; 6:19. Doi:10.21037/sci.2019.06.04

Response: We have improved the introduction, and added the hypothesis on page 4 line 13-15. We have also referred your suggested reference on page 3 line 13-15 as reference number 1.

2)Which passage of cells were used? Please specify in the text of manuscript.

Response: We have added the cell passage used and specify it on the method section on page 4 line 23 and page 5 line 7.

3)Generally, methods section must be cited with related references.

Response: We have cited with related references as suggested on materials and methods section and added them in the reference list.

4) *The identification and characterization method of isolated mesenchymal stem cells must be discussed in detail. This comment is required. You can use and refer the following papers which explained elaborately and completely the "flow cytometric analysis and multi-lineage differentiation method for characterization of mesenchymal stem cells" in "Preparation of HUCMSCs and Calcium Hydroxide" section:*

• *Interleukin-6, -8, and TGF- β Secreted from Mesenchymal Stem Cells Show Functional Role in Reduction of Telomerase Activity of Leukemia Cell Via Wnt5a/ β -Catenin and P53 Pathways.*

• *L-carnitine significantly decreased aging of rat adipose tissue-derived mesenchymal stem cells. Veterinary research communications. 2017 Mar 1;41(1):41-7.*

Response: We have used and referred the above-mentioned papers as suggested as reference number 9 and 10 on page 12.

5) *Also, the immunocytochemistry method must be explained in detail. You can use and refer the following paper which explained and analyzed elaborately the "immunocytochemistry method" in "Evaluation of apoptosis" section:*

• *Mesenchymal Stem Cells Could Be Considered as a Candidate for Further Studies in Cell-Based Therapy of Alzheimer's Disease via Targeting the Signaling Pathways. ACS Chemical Neuroscience. 2020 Apr 20;11(10):1424-35.*

Response: We have explained the method in detail using FITC method, and the suggested above reference has been added to the manuscript on page 6.

6) *Also, since the discussion section is one of the most important parts of the paper, this section must be improved with more attention and explanation. In the discussion section, results must be compared with another results from previous studies.*

Response: We have added the discussion and another result from previous study has been added on page 10 line 15-20.

Thank you once again. We hope that our response would be sufficient and acceptable for you.

Best regards,

Eric Priyo Prasetyo & team.

1 ORIGINAL RESEARCH

2 **Calcium Hydroxide Increases Human Umbilical Cord**
3 **Mesenchymal Stem Cells Expressions of Apoptotic**
4 **Protease-Activating Factor-1, Caspase-3 and Caspase-9**

5 Eric Priyo Prasetyo^{1,2}, Mefina Kuntjoro^{1,3}, Setyabudi Goenharto², Devi Eka Juniarti², Febriastuti
6 Cahyani², Nike Hendrijantini³, Alexander Patera Nugraha⁴, Ninuk Hariyani⁵, Fedik Abdul Rantam^{6,7}

7

8 ¹Doctoral Program, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

9 ²Department of Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga,
10 Surabaya, Indonesia.

11 ³Department of Prosthodontics, Faculty of Dental Medicine, Universitas Airlangga, Surabaya,
12 Indonesia.

13 ⁴Department of Orthodontics, Faculty of Dental Medicine, Universitas Airlangga, Surabaya,
14 Indonesia.

15 ⁵Department of Dental Public Health, Faculty of Dental Medicine, Universitas Airlangga, Surabaya,
16 Indonesia.

17 ⁶Stem Cell Research and Development Center, Universitas Airlangga, Surabaya, Indonesia.

18 ⁷Laboratory of Virology, Department of Microbiology and Immunology, Faculty of Veterinary
19 Medicine, Universitas Airlangga, Surabaya, Indonesia.

1

2 **Correspondence:**

3 Eric Priyo Prasetyo

4 Department of Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga, Jalan
5 Mayjend. Prof. Dr. Moestopo 47, Surabaya, Indonesia.

6 Tel/Fax +62 31 5030255

7 E-mail: eric-p-p@fkg.unair.ac.id

8

9 **Abstract:**

10 **Purpose:** Calcium hydroxide is a gold standard dental material generally used for pulpal and
11 periapical therapy including regenerative endodontic procedures **because of its positive properties.**
12 **However, evaluation about this material on stem cells is limited.** Human umbilical cord
13 mesenchymal stem cells (HUCMSCs) are potential to be used in regenerative therapy.
14 Regenerative therapy needs a sustainable cell supply to maintain its regenerative capacity. The
15 aim of this study was to ascertain the apoptosis result of calcium hydroxide on HUCMSCs through
16 the expression of Apoptotic Protease-Activating Factor-1 (APAF-1), Caspase-3, and Caspase-9.

17 **Materials and Methods:** This study used a thawed frozen stock of passage 5 HUCMSCs, grown in
18 Minimum Essential Medium (MEM) Alpha containing calcium hydroxide at concentration of 0.1
19 microgram/mL for 1, 3 and 7 days. **Polyclonal antibody with fluorescence isothiocyanate (FITC)**
20 **label was used to evaluate the expressions.** APAF-1, Caspase-3, and Caspase-9 expressions were
21 recorded and compared on every observation day using **fluorescence microscope.** Analysis of

1 variance was performed to analyze the significance among the results of treatment groups. The
2 results were concluded significant if $p < 0.05$.

3 **Results:** The addition of calcium hydroxide in MEM Alpha medium increases HUCMSCs
4 expression of APAF-1, caspase-3 and caspase-9 significantly, compared to the control group
5 without calcium hydroxide ($p < 0.05$) in all the times. Day 1 showed the lowest increase followed by
6 higher expressions on day 3 and day 7.

7 **Conclusion:** HUCMSCs express increased APAF-1, caspase-3 and caspase-9 after in-vitro
8 calcium hydroxide exposure. This should be considered when using calcium hydroxide on
9 HUCMSCs for regenerative procedures with regard to other positive properties.

10 **Keywords:** apoptosis, calcium hydroxide, caspases, mesenchymal stem cells, umbilical cord

11

12 Introduction

13 Mesenchymal stem cells (MSC) are the most commonly used for regenerative therapy. These cells
14 can easily be isolated, transplanted and It has the capacity of immune regulatory, self-renewal and
15 differentiation into many cell types, such as osteocyte, neurons, adipocyte and chondrocyte.¹

16 Human umbilical cord mesenchymal stem cells (HUCMSCs) are MSC derived from Wharton's jelly.
17 HUCMSCs may be an appealing contender for application in periapical, pulpal, and alveolar bone
18 regeneration.² With regard to other adult stem cell sources, HUCMSCs are primordial, involving
19 non-invasive collection method, higher differentiation capacity, immunosuppressive, immune
20 privileged, and rich in stemness characteristics.^{3,4}

21 Calcium hydroxide has been used in a number of treatment protocols in endodontics, including
22 inter-appointment dressing, pulp capping agents, and root canal sealers.⁵ Calcium hydroxide is a

1 vital alkaline substance administered in a favorable protocol of regenerative therapy.⁶ Calcium
2 hydroxide may supply calcium ions that are necessary in the outset of intrinsic apoptotic pathway.
3 Stem cell availability and sustainability is important for the regenerative process in regenerative
4 procedures, including regenerative endodontic procedures. The availability and persistence of
5 HUCMSCs can be affected by programmed cell death or apoptosis. Previous study showed that
6 stem cells reactions may vary based on time variation of contact towards the presence of calcium
7 hydroxide.⁷ Apoptosis is crucial for tissue development and regeneration.⁸ Apoptotic protease-
8 activating factor-1 (APAF-1), caspase-3, and caspase-9 are among prominent markers for
9 apoptosis. In order to understand the effect of calcium hydroxide on HUCMSCs' survival, we
10 observed the expression of these apoptotic biomarkers on HUCMSCs. Therefore, the aim of this
11 study was to explore the expression of apoptotic protease-activating factor-1 (APAF-1), caspase-3,
12 and caspase-9 on HUCMSCs after continuous contact with calcium hydroxide for 1 day, 3 days,
13 and 7 days period in vitro. The hypothesis of this study is calcium hydroxide would increase
14 apoptotic protease-activating factor-1 (APAF-1), caspase-3, and caspase-9 expressions on
15 HUCMSCs after continuous contact for 1 day, 3 days, and 7 days period in vitro.

16

17 **Materials and Methods**

18 ***Preparation of HUCMSCs and Calcium Hydroxide***

19 Ethical clearance for this study was given by the Commission of Ethical Clearance for Health
20 Research from Faculty of Dental Medicine Universitas Airlangga, Surabaya, Indonesia (No.
21 059/HRECC.FODM/II/2020). This study was conducted following ethical standards of experiments.
22 The cell donors provided informed consent, in accordance with the Declaration of Helsinki. Frozen
23 HUCMSCs stock from passage 5 was supplied by the Stem Cell Research and Development

1 Center Universitas Airlangga, Surabaya, Indonesia. This frozen stock was previously isolated and
2 characterized.

3 HUCMSCs was confirmed previously by conducting flow cytometric check utilizing FACS Calibur
4 (BD Biosciences, USA) for specific antibodies purchased from Becton-Dickinson (BD Biosciences,
5 USA) for positive CD73, CD90, CD105, and negative CD45 and CD34. This fluorescence activated
6 cell sorter (FACS) instrument was utilized to count the fluorescence intensity expressed by the
7 cells.^{9,10} Frozen stock of passage 5 HUCMSCs was taken from minus 80°C cold storage and
8 thawed by water bathing. After thawing, HUCMSCs were transported to other container with 10 mL
9 minimum essential medium (MEM) alpha (Gibco, UK) at 37°C and centrifuged until they formed a
10 cell pellet at 1600 rpm for 5 minutes. The cell pellet was resuspended in 12 mm culture plate
11 containing MEM alpha medium and stored in incubator for several hours at 37°C. The thawed
12 HUCMSCs were expanded and ready to be used for further study.¹¹

13 Calcium hydroxide was obtained by combining minimum essential medium alpha (Gibco, UK) with
14 the powder (EMSURE Merck, Germany). Calcium hydroxide concentration of 0.1 microgram/mL
15 was used in this experiment.

16

17 ***Evaluation of Apoptosis***

18 HUCMSCs were assigned into 9 groups of control and 9 groups of calcium hydroxide treatment,
19 with allocation for 1, 3, and 7 days of observations. Each group consisted of six wells of M24 plate
20 (Iwaki Asahi, Japan). Every M24 plate well was seeded with 250.000 HUCMSCs in 1 mL media.
21 HUCMSCs in the treatment groups were grown in minimum essential medium (MEM) alpha
22 containing 0.1 microgram/mL calcium hydroxide, and the control groups were grown in MEM alpha
23 medium only without the addition of calcium hydroxide. The groups were cultured in incubator at
24 37°C and 5% CO₂ and observed for 1, 3, and 7 days.

1 Apoptosis reaction of HUCMSCs was investigated through the expressions of apoptotic protease-
2 activating factor-1 (Bioss Antibodies, USA), Caspase-3 (Bioss Antibodies, USA), and Caspase-9
3 (Bioss Antibodies, USA). The investigation was done following the manufacturer's instructions
4 Other previous study used flow cytometric analysis for assessments.¹² In this research APAF-1,
5 Caspase-3, and Caspase-9 expressions were assessed for apoptosis using polyclonal antibody
6 with fluorescence isothiocyanate (FITC) label (Bioss Antibodies, USA). Observation of the results
7 was done with fluorescence microscope (Olympus, Japan) with imaging system at 100x
8 magnification and processed in ImageJ software for fluorescence quantification (National Institute
9 of Health, USA).¹³

10

11 ***Statistical Analysis***

12 The evaluation was conducted in triplicates. Data were expressed as mean \pm standard deviation
13 from the experiment. The data were checked for normal distribution. T-test was carried out for
14 comparisons between treatment and control groups. One-way analysis of variance (ANOVA) was
15 used for comparisons of three groups among day 1, 3, and 7 on each expression. Difference
16 among groups was appraised significant if $P < 0.05$.

17

18 **Results**

19 Mean and standard deviation (SD) of APAF-1, Caspase-3, and Caspase-9 expressions from
20 HUCMSCs in control groups and calcium hydroxide groups is available on Table 1. Calcium
21 hydroxide increase APAF-1 expression, corresponds to the days observed (Figure 1). APAF-1
22 expression was low on day 1 of both control and calcium hydroxide groups. Both control and
23 calcium hydroxide groups showed significant increase ($p < 0.05$) from day 1 to day 3, and then the

1 calcium hydroxide group increase significantly on day 7 compared to the control group. There was
2 a significant increase of APAF-1 expression on both the control and calcium hydroxide treatment
3 groups from day 1 to day 3, and from day 3 to day 7 ($p < 0.05$). Significance (p value) among
4 exposure day in the control groups and calcium hydroxide groups on APAF-1 expression is
5 available in Table 2.

6 Calcium hydroxide increase caspase-3 expression, corresponds to the days observed (Figure 1).
7 Caspase-3 expression of the control group was low on day 1, day 3, and day 7. Caspase-3
8 expression of calcium hydroxide group increase significantly on day 3 and then slightly decrease
9 on day 7 (there was no significant decrease ($p > 0.05$) between day 3 and day 7). There was no
10 significant difference of Caspase-3 expression on day 1. There was significant difference between
11 the control groups and calcium hydroxide groups on day 3 and day 7 ($p < 0.05$). Significance (p
12 value) among exposure day in the control groups and calcium hydroxide groups on caspase-3
13 expression is available in Table 3.

14 Calcium hydroxide increase Caspase-9 expression, corresponds to the days observed (Figure 1).
15 Caspase-9 expression of both the control groups and calcium hydroxide groups were low on day 1.
16 The control group showed a gradual increase from day 1 to day 7. The calcium hydroxide groups
17 revealed a significant increase of Caspase-9 on day 3 and then quite steady on day 7 without
18 significance ($p > 0.05$). Significance (p value) among exposure day in the control groups and
19 calcium hydroxide groups on caspase-9 expression is available in Table 4.

20

21 Discussion

22 There are many sources of mesenchymal stem cells. Among other stem cells, HUCMSCs is
23 prominent because the isolation process is non-invasive and they can be expanded in large
24 quantities.¹⁴ HUCMSCs and its application in regenerative dental procedures are limited. In this

1 study, the HUCMSCs are according to standardization of MSCs cluster of differentiation surface
2 markers and morphology.¹⁵ The HUCMSCs were grown in minimum essential medium alpha with
3 and without the addition of calcium hydroxide, under normal culture condition. MTT assay was
4 used in this study to measure cell viability under the effect of calcium hydroxide, and concentration
5 of 0.1 microgram/mL was chosen because it has more than 60% viable cells. In this study we found
6 that 0.1 microgram/mL of calcium hydroxide promotes the expressions of APAF-1, caspase-3 and
7 caspase-9.

8 Apoptosis is a usual form of programmed cell death process that participates in homeostatic control
9 of cell population, without inflammation take place.¹⁶ Apoptosis is sophisticatedly managed and
10 balanced mechanism which helps in the elimination of unfavorable cells throughout every
11 organism's life cycle.¹⁷ Apoptosis is initiated by an intrinsic (classical or mitochondrial) pathway and
12 an extrinsic (death receptor or cytoplasmic) pathway.¹⁸ Both pathways converge in the final
13 apoptotic execution phase, which is marked by nuclear DNA breakdown, protein cleavage, and
14 apoptotic cell identification by phagocytic cells.¹⁹

15 Any condition or stimuli to a cell's environment is capable to induce apoptotic signaling.²⁰ Cells
16 experiencing apoptosis release extracellular vesicles, such as apoptotic bodies, micro-vesicles,
17 and apoptotic exosomes, which roles in immune responses and inflammation, as an active
18 communication from dying cells to surrounding living cells.¹⁷ Defected or dysregulation in apoptotic
19 pathways may lead towards various malignancies and diseases, including AIDS, diabetes, and
20 neurodegenerative diseases involving the perturbation of genes.²¹

21 Apoptosis is influenced by many signals interdependently. In intrinsic apoptotic pathway there are
22 other influencing factors, such as the effector proteins BAK and BAX, antiapoptotic proteins (A1,
23 Mcl-1, Bcl-xL, Bcl-w, and Bcl-2) and proapoptotic BH3-only proteins (Puma, Noxa, Bid, Bad, Bik,
24 Bmf, Bim, and Hrk).²² Active BAX and BAK induce mitochondrial outer-membrane permeabilization
25 (MOMP), and initiates cytochrome c efflux, which form a complex with APAF-1 and activate

1 caspase-9 to activate caspase-3.²³ In extrinsic apoptotic pathway there are influencing factors,
2 such as TNF receptor superfamily (TNF alpha, FAS and TRAIL receptors), cytosolic death domains
3 (DD) receptors to bind TNFR-associated death domain (TRADD) or Fas-associated death domain
4 (FADD), and finally form a death-inducing signaling complex (DISC), which would activate
5 caspase-3, caspase-6 and caspase-7.¹⁷

6 APAF-1 (Apoptotic protease activating factor 1) is normally present in cytoplasm in inactive form,
7 and can be activated by cytochrome c and controlled by pro and anti-apoptotic molecules.¹⁹ APAF-
8 1 is responsible for caspase-9 activation. Aside from its central role in the initiation of cell death,
9 APAF-1 have non-apoptotic functions. The non-apoptotic roles of APAF-1 are modulatory effect on
10 cell cycle during DNA damage induced by genotoxic stress and participation in the cytoskeleton
11 arrangement and centrosomic microtubule nucleation process.²⁴

12 Caspases are a collection of proteases recognized for their important function in programmed cell
13 death, abbreviated from cysteine-aspartic protease activity.¹⁹ Caspases have been grouped into
14 apoptotic caspases and inflammatory caspases. According to the action mechanism, apoptotic
15 caspases are classified into initiator caspases and executioner caspases. Caspase-3 and caspase-
16 9 belong to apoptotic caspase group; caspase-3 is executioner and caspase-9 is initiator.¹⁷

17 Caspase-3, a pivotal role in apoptosis, is a downstream effector and is affected by intrinsic and
18 extrinsic pathways.^{25,26} Various stimuli can affect the apoptosis. Calcium ion plays a crucial role in
19 the intrinsic apoptotic pathway: high regulation of calcium into mitochondria lead to the deliverance
20 of cytochrome c.¹⁶ In the cytoplasm, cytochrome c ties to a cytoplasmic protein known as APAF-1
21 and shapes the apoptosome that initiates procaspase-9 into caspase-9 and then trigger other
22 caspases that will eventually execute cell disintegration.²⁷

23 Increased expression of APAF-1 would mean increased levels of apoptosis. Caspase 9 would turn
24 on pro-caspase-3 into caspase-3 as caspase effector which convey apoptosis.²⁸ The quantity of

1 APAF-1 and procaspase-9 affect the proportion of caspase-9 homodimers and heterodimers
2 shaped in apoptosome.²⁹

3 Previous reports demonstrated that mitochondrial caspases in intrinsic apoptosis have several non-
4 apoptotic roles, including cellular reprogramming, differentiation, immunogenic, and proliferation.²³
5 Most caspases have roles in cell proliferation, survival, inflammation or differentiation.²⁵ Other than
6 lethal function in apoptosis, caspase-3 and caspase-9 have non-lethal function in cell
7 differentiation, with caspase-3 have more functions in cell maturation and activation.²³ Caspase-3
8 has vital role in tissue regeneration, differentiation, and neural development differently and not
9 involving apoptotic activity.³⁰

10 In this study, the addition of calcium hydroxide was correlated with high expression of APAF-1,
11 caspase-3, and caspase-9, but these intense expressions are also happened to the control groups.
12 HUCMSCs experience apoptosis on its own timing, but the addition of calcium hydroxide
13 accelerated HUCMSCs to apoptosis. Calcium hydroxide trigger the mitochondrial apoptosis. This
14 trigger might not only cause apoptosis but might also has a function in immune defense
15 mechanism.¹⁷ The increased apoptosis in this study may imply clinical practice during regenerative
16 endodontic procedures. In this case, it may have roles in stem cell proliferation, survival or
17 differentiation. Other study involving calcium hydroxide provided that it upregulates HUCMSCs
18 interleukin-10 expression and osteogenic differentiation.³¹ As this research is an in vitro study,
19 there are limitations. Therefore, we should also explore at broader potential aspects or biomarkers
20 other than apoptosis.

21 However, apoptosis is needed for homeostasis and pathological processes.²² Previous reports
22 showed that during apoptosis, cells sustain the integrity of the plasma membrane to prevent
23 inflammation.⁸ This study indicate evidence regarding HUCMSCs apoptosis induced by calcium
24 hydroxide. Appropriate apoptosis of HUCMSCs is advantageous for tissue regeneration.

25

1 **Conclusion**

2 In conclusion, although HUCMSCs has a high proliferation capacity and anti-inflammation capacity,
3 this study provide evidence that calcium hydroxide promotes HUCMSCs to apoptosis, even if it was
4 administered in non-toxic dose. Further studies are needed to know more about HUCMSCs use in
5 regenerative dental procedures.

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9 the given technical supports.

10 **Disclosure**

11 The authors report no conflicts of interest in this work.

12

13 **References**

- 14 1. Rajabzadeh N, Fathi E, Farahzadi R. Stem cell-based regenerative medicine. *Stem Cell*
15 *Investigation*. 2019; 6:19. DOI: 10.21037/sci.2019.06.04.
- 16 2. Meguid EA, Ke Y, Ji J, El-Hashash AHK. Stem cells applications in bone and tooth repair
17 and regeneration: New insights, tools, and hopes. *J Cell Physiol*. 2017; 9999:1-11. DOI:
18 10.1002/jcp.25940.

- 1 3. Li T, Xia M, Gao Y, Chen Y, Xu Y. Human umbilical cord mesenchymal stem cells: an
2 overview of their potential in cell-based therapy. *Expert Opin Biol Ther.* 2015; 15:1293-
3 1306. DOI: 10.1517/14712598.2015.1051528.
- 4 4. Subramanian A, Fong CY, Biswas A, Bongso A. Comparative characterization of cells from
5 the various compartments of the human umbilical cord shows that the Wharton's jelly
6 compartment provides the best source of clinically utilizable mesenchymal stem cells.
7 *PLoS ONE.* 2015; 10(6): e0127992. DOI: 10.1371/journal.pone.0127992.
- 8 5. Ba-Hattab R, Al-Jamie M, Aldreib H, Alessa L, Alonazi M. Calcium hydroxide in
9 endodontics: an overview. *Open journal of stomatology.* 2016; 6: 274-289. DOI:
10 10.4236/ojst.2016.612033.
- 11 6. Kahler B, Chugal N, Lin LM. Alkaline materials and regenerative endodontics: a review.
12 *Materials.* 2017; 10: 1389. DOI: 10.3390/ma10121389.
- 13 7. Prasetyo EP, Widjiastuti I, Cahyani F, Kuntjoro M, Hendrijantini N, Hariyani N, Winoto ER,
14 Nugraha AP, Goenharto S, Susilowati H, Hendrianto E, Rantam FA. Cytotoxicity of calcium
15 hydroxide on human umbilical cord mesenchymal stem cells. *Pesqui Bras Odontopediatria*
16 *Clin Integr* 2020; 20: e0044. DOI: 10.1590/pboci.2020.149
- 17 8. Wu T, Li L, Du R, Jiang L, Zhu Y. Hydrogen peroxide induces apoptosis in human dental
18 pulp cells via caspase-9 dependent pathway. *Journal of Endodontics.* 2013; 39(9): 1151-
19 1155. DOI: 10.1016/j.joen.2013.06.006.
- 20 9. Fathi E, Valipour B, Sanaat Z, Charoudeh HN, Farahzadi R. Interleukin-6, -8, and TGF- β
21 secreted from mesenchymal stem cells show functional role in reduction of telomerase
22 activity of leukemia cell via Wnt5a/b-catenin and P53 pathways. *Adv Pharm Bull.* 2020;
23 10(2): 307-314. DOI: 10.34172/apb.2020.037.
- 24 10. Mobarak H, Fathi E, Farahzadi R, Zarghami N, Javanmardi S. L-carnitine significantly
25 decreased aging of rat adipose tissue-derived mesenchymal stem cells. *Vet Res Commun.*
26 2017; 41: 41-47. DOI: 10.1007/s11259-016-9670-9.

- 1 11. Kuntjoro M, Prasetyo EP, Cahyani F, Kamadjaja MJK, Hendrijantini N, Laksono H,
2 Rahmania PN, Ariestania V, Nugraha AP, Ihsan IS, Dinartyanti A, Rantam FA.
3 Lipopolysaccharide's cytotoxicity on human umbilical cord mesenchymal stem cells.
4 *Pesqui Bras Odontopediatria Clin Integr* 2020; 20: e0048. DOI: 10.1590/pboci.2020.153.
- 5 12. Farahzadi R, Fathi E, Vietor I. Mesenchymal stem cells could be considered as a
6 candidate for further studies in cell-based therapy of Alzheimer's disease via targeting the
7 signaling pathways. *ACS Chem Neurosci*. 2020; 11: 1424-1435. DOI:
8 10.1021/acscchemneuro.0c00052.
- 9 13. Jensen EC. Quantitative analysis of histological staining and fluorescence using ImageJ.
10 *Anatomical Record*. 2013; 296:378-381. DOI: 10.1002/ar.22641.
- 11 14. Deng Y, Zhang Y, Ye L, Zhang T, Cheng J, Chen G, Zhang Q, Yang Y. Umbilical cord-
12 derived mesenchymal stem cells instruct monocytes towards an IL10-producing phenotype
13 by secreting IL6 and HGF. *Sci Rep*. 2016; 6: 37566. DOI: 10.1038/srep37566.
- 14 15. Nugraha AP, Prasetyo EP, Kuntjoro M, Ihsan IS, Dinartyanti A, Susilowati H, Hendrianto E,
15 Narmada IB, Ernawati DS, Nugraha AP, Rantam FA. The effect of cobalt (II) chloride in the
16 viability percentage and the induced hypoxia inducible factor-1a of human adipose
17 mesenchymal stem cells (HAMSCs): an in vitro study. *Sys Rev Pharm*. 2020; 11(6): 308-
18 314. DOI: 10.31838/srp.2020.6.49.
- 19 16. Kontogiannis TG, Tosios KI, Kerezoudis NP. Effect of calcium hydroxide as intracanal
20 medicament on the expression of caspase-9 located within the radicular cyst epithelium.
21 *Australian Endodontic Journal*. 2019; 1-5. DOI: 10.1111/aej.12325.
- 22 17. Kakarla R, Hur J, Kim YJ, Kim J, Chwae Y. Apoptotic cell-derived exosomes: messages
23 from dying cells. *Experimental & Molecular Medicine*. 2020; 52:1-6. DOI: 10.1038/s12276-
24 019-0362-8.
- 25 18. Rogers C, Fernandes-Alnemri T, Mayes L, Alnemri D, Cingolani G, Alnemri ES. Cleavage
26 of DFNA5 by caspase-3 during apoptosis mediates progression to secondary necrotic /

- 1 pyroptotic cell death. *Nature Communications*. 2017; 8: 14128. DOI:
2 10.1038/ncomms14128.
- 3 19. Cavalcante GC, Schaan AP, Cabral GF, Santana-da-Silva MN, Pinto P, Vidal AF, Ribeiro-
4 dos-Santos A. A cell's fate: an overview of the molecular biology and genetics of apoptosis.
5 *International Journal of Molecular Sciences*. 2019; 20: 4133. DOI: 10.3390/ijms20174133.
- 6 20. Brokatzky D, Dorflinger B, Haimovici A, Weber A, Kirschnek S, Vier J, Metz A, Henschel J,
7 Steinfeldt T, Gentle IE, Hacker G. A non-death function of the mitochondrial apoptosis
8 apparatus in immunity. *The EMBO Journal*. 2019; 38: e100907. DOI:
9 10.15252/embj.2018100907.
- 10 21. Arif M, Syed A, Mahmood A, Khan S, Rizwan M, Munir A. Modelling of apoptosis through
11 gene interaction network and analysis of gene expression pattern. *Meta Gene*. 2020; 25:
12 100730. DOI: 10.1016/j.mgene.2020.100730.
- 13 22. Lamb HM. Double agents of cell death: novel emerging functions of apoptotic regulators.
14 *The FEBS Journal*. 2020; 287: 2647-2663. DOI: 10.1111/febs.15308.
- 15 23. McArthur K, Kile BT. Apoptotic caspases: multiple or mistaken identities. *Trends in Cell*
16 *Biology*. 2018; 28(6): 475-493. DOI: 10.1016/j.tcb.2018.02.003.
- 17 24. Shakeri R, Kheirollahi A, Davoodi J. Apaf-1: regulation and function in cell death.
18 *Biochimie*. 2017; 135: 111-125. DOI: 10.1016/j.biochi.2017.02.001.
- 19 25. Aydogan A, Kocer G, Ozmen O, Kocer M, Onal L, Koskan O. Immunohistochemical
20 expression of caspase-3, caspase-5, caspase-7 and apoptotic protease-activating factor-1
21 (APAF-1) in the liver and kidney of rats exposed to zoledronic acid (ZOL) and basic
22 fibroblast growth factor (bFGF). *Veterinary Quarterly*. 2014; 34(3): 137-142. DOI:
23 10.1080/01652176.2014.928759.
- 24 26. Pradeep AR, Suke DK, Prasad MVR, Singh SP, Martande SS, Nagpal K, Naik SB,
25 Guruprasad CN, Raju AP, Singh P, Siddaya M. Expression of key executioner of apoptosis
26 caspase-3 in periodontal health and disease. *Journal of Investigative and Clinical Dentistry*.
27 2016; 7: 174-179. DOI: 10.1111/jicd.12134.

- 1 27. Taylor RC, Cullen SP, Martin SJ. Apoptosis: controlled demolition at the cellular level. Nat
2 Rev Mol Cell Biol. 2008; 9: 231-41.
- 3 28. Nandarani RE, Widjiastuti I, Mooduto L. Pulp fibroblast cell apoptosis after application of
4 hema dentine bonding material with ethanol and water solvent. Brazilian Dental Journal.
5 2019; 30(3): 208-212. DOI: 10.1590/0103-6440201902524.
- 6 29. Wu C, Lee S, Malladi S, Chen M, Mastrandrea NJ, Zhang Z, Bratton SB. The apaf-1
7 apoptosome induces formation of caspase-9 homo- and heterodimers with distinct
8 activities. Nature Communications. 2016; 7: 13565. DOI: 10.1038/ncomms13565.
- 9 30. Shalini S, Dorstyn L, Dawar S, Kumar S. Old, new and emerging functions of caspases.
10 Cell Death and Differentiation. 2015; 22: 526-539. DOI: 10.1038/cdd.2014.216.
- 11 31. Prasetyo EP, Kuntjoro M, Cahyani F, Goenharto S, Saraswati W, Juniarti DE, Hendrijantini
12 N, Hariyani N, Nugraha AP, Rantam FA. Calcium hydroxide upregulates interleukin-10
13 expression in time dependent exposure and induces osteogenic differentiation of human
14 umbilical cord mesenchymal stem cells. Int J Pharm Res. 2021; 13(1): 140-145. DOI:
15 10.31838/ijpr/2021.13.01.023.

16 **Figure Legend:**

17 **Figure 1** HUCMSCs culture seen under light microscope (L) and fluorescence microscope (F)
18 showing the expressions of APAF-1, Caspase-3, and Caspase-9 observed on day 1, day 3, and
19 day 7 between control groups and calcium hydroxide (CH) groups.
20

21 **Table Legend:**

22 **Table 1** Mean and Standard Deviation (SD) of APAF-1, Caspase-3, and Caspase-9 expressions
23 from HUCMSCs in control groups and calcium hydroxide groups.
24

25 **Table 2** Significance (*p* value) among exposure day in the control groups and calcium hydroxide
26 groups on APAF-1 expression.
27

28 **Table 3** Significance (*p* value) among exposure day in the control groups and calcium hydroxide
29 groups on Caspase-3 expression.
30

31 **Table 4** Significance (*p* value) among exposure day in the control groups and calcium hydroxide
32 groups on Caspase-9 expression.
33



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