BUKTI KORESPONDENSI
JURNAL INTERNASIONAL BEREPUTASI TERAKREDITASI Q3

Judul Artikel : **(C05)** Lipopolysaccharide's cytotoxicity on human umbilical cord mesenchymal stem cells

Jurnal : Pesquisa Brasileira em Odontopediatria e Clínica Integrada

Penulis : Mefina Kuntjoro, Eric Priyo Prasetyo, Febriastuti Cahyani, ***Michael Josef Kridanto Kamadjaja****, Nike Hendrijantini, Harry Laksono, Primanda Nur Rahmania, Vivin Ariestania, Alexander Patera Nugraha, Igo Syaiful Ihsan, Aristika Dinaryanti, Fedik Abdul Rantam

No	Perihal	Tanggal	Halaman
1	Bukti submit dan artikel yang disubmit	10 Maret 2020	14
2	Bukti Revisi 1	23 April 2020	1-3
3	Bukti Revisi 2	23 Mei 2020	4-8
4	Bukti Revisi 3		
5	Decision 1		
6	Decision 2		
7	Decision 3		
8	Bukti accepted	28 Mei 2020	9-15
9	Bukti published		

Pesquisa Brasileira em Odontopediatria e Clínica Integrada

Decision Letter (PBOCI-2020-0048)

From: alessandrouepb@gmail.com

To: michael-j-k-k@fkg.unair.ac.id

CC:

Subject: Pesquisa Brasileira em Odontopediatria e Clínica Integrada - Decision on Manuscript ID PBOCI-2020-0048

Body: 23-Apr-2020

Dear Dr. Kamadjaja:

Manuscript ID PBOCI-2020-0048 entitled "Lipopolysaccharide's Cytotoxicity on Human Umbilical Cord Mesenchymal Stem Cells" which you submitted to the Pesquisa Brasileira em Odontopediatria e Clínica Integrada, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.

The reviewer(s) have recommended consideration for publication, but also suggested some revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript, in order for it to be able to be considered for acceptance.

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Once again, thank you for submitting your manuscript to the Pesquisa Brasileira em Odontopediatria e Clínica Integrada and I look forward to receiving your revision.

Sincerely,
Prof. Alessandro Cavalcanti
Editor-in-Chief, Pesquisa Brasileira em Odontopediatria e Clínica Integrada

alessandrouepb@gmail.com

Associate Editor
Comments to the Author:
Please consider the recommendations of both reviewers

Entire Scoresheet:
Reviewer: 1

Recommendation: Minor Revision

Comments:
Please address the comments as indicated using annotations in the attachment.

Additional Questions:
Does the manuscript contain new and significant information to justify publication?: Yes

Does the Abstract (Summary) clearly and accurately describe the content of the article?: Yes

Is the problem significant and concisely stated?: Yes

Are the methods described comprehensively?: No

Are the interpretations and conclusions justified by the results?: Yes

Is adequate reference made to other work in the field?: Yes

Length of article is: Adequate

Number of tables is: Adequate

Number of figures is: Adequate

Please state any conflict(s) of interest that you have in relation to the review of this paper (state "none" if this is not applicable).: NONE

Rating:

Interest: 1. Excellent

Quality: 2. Good

Originality: 1. Excellent

Overall: 2. Good

Reviewer: 2

Recommendation: Major Revision

Comments:
Presented study provides information about the lipopolysaccharide's cytotoxicity on human umbilical cord mesenchymal stem cells. The manuscript is not well prepared and needs to be improved. Experimental procedure (cytotoxicity detection) is limited only to MTT method, and at least one additional confirmatory method is necessary. (Also, deeper insides in mechanisms of cytotoxicity could be implemented in this study.) Within experiments only three parallel measurements were conducted, for more reliable statistical analysis more data is needed. If the authors are willing to implement the above suggestions in their study, the paper may be considered to be published after a major revision. English language needs improvement.

Additional Questions:
Does the manuscript contain new and significant information to justify publication?: No

Does the Abstract (Summary) clearly and accurately describe the content of the article?: Yes

Is the problem significant and concisely stated?: No

Are the methods described comprehensively?: Yes

Are the interpretations and conclusions justified by the results?: Yes

Is adequate reference made to other work in the field?: Yes

Length of article is: Adequate

Number of tables is: Adequate

Number of figures is: Adequate

Please state any conflict(s) of interest that you have in relation to the review of this paper (state "none" if this is not applicable).: No conflict of interest.

Rating:

Interest: 3. Average

Quality: 4. Below Average

Originality: 3. Average

Overall: 3. Average

Date Sent: 23-Apr-2020

File 1: - PBOCI-2020-0048-1.pdf

File 2: - PBOCI-2020-0048_Proof_hi.pdf

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Decision Letter (PBOCI-2020-0048.R1)

From: alessandrouepb@gmail.com

To: michael-j-k-k@fkg.unair.ac.id

CC:

Subject: Pesquisa Brasileira em Odontopediatria e Clínica Integrada - Decision on Manuscript ID PBOCI-2020-0048.R1

Body: 23-May-2020

Dear Dr. Kamadjaja:

Manuscript ID PBOCI-2020-0048.R1 entitled "Lipopolysaccharide's Cytotoxicity on Human Umbilical Cord Mesenchymal Stem Cells" which you submitted to the Pesquisa Brasileira em Odontopediatria e Clínica Integrada, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.

The reviewer(s) have recommended consideration for publication, but also suggested some revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript, in order for it to be able to be considered for acceptance.

To revise your manuscript, log into https://mc04.manuscriptcentral.com/pboci-scielo and enter your Author Center, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.

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Once the revised manuscript is prepared, you can upload it and submit it through your Author Center.

When submitting your revised manuscript, you will be able to respond to the comments made by the reviewer(s) in the space provided. You can use this space to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the reviewer(s).

IMPORTANT: Your original files are available to you when you upload your revised manuscript. Please delete any redundant files before completing the submission.

Because we are trying to facilitate timely publication of manuscripts submitted to the Pesquisa Brasileira em Odontopediatria e Clínica Integrada, your revised manuscript should be submitted by 23-Jun-2020. If it is not possible for you to submit your revision by this date, we may have to consider your paper as a new submission.

Once again, thank you for submitting your manuscript to the Pesquisa Brasileira em Odontopediatria e Clínica Integrada and I look forward to receiving your revision.

Sincerely,
Prof. Alessandro Cavalcanti
Editor-in-Chief, Pesquisa Brasileira em Odontopediatria e Clínica Integrada

alessandrouepb@gmail.com

Associate Editor
Comments to the Author:
(There are no comments.)

Entire Scoresheet:
Reviewer: 1

Recommendation: Minor Revision

Comments:
The authors have carried out most of the corrections. However, they still have not addressed few comments as indicated earlier. The authors are suggested to read the comments carefully and address accordingly.
1. Why were these particular concentrations selected? Is it based on previous study or literature? (PS concentrations of 0.39, 0.78, 1.56, 31.25, 6.25, 12.5, and 25 μg/mL were to be compared in this experiment)
2. What about dissolving the crystals in MTT assay? Usually, DMSO is added for this purpose. The authors mention that they measure the crystals. Not clear. Please clarify.

Additional Questions:
Does the manuscript contain new and significant information to justify publication?: Yes

Does the Abstract (Summary) clearly and accurately describe the content of the article?: Yes

Is the problem significant and concisely stated?: Yes

Are the methods described comprehensively?: No

Are the interpretations and conclusions justified by the results?: Yes

Is adequate reference made to other work in the field?: Yes

Length of article is: Adequate

Number of tables is: Adequate

Number of figures is: Adequate

Please state any conflict(s) of interest that you have in relation to the review of this paper (state "none" if this is not applicable).: None

Rating:

Interest: 1. Excellent

Quality: 1. Excellent

Originality: 1. Excellent

Overall: 2. Good

Reviewer: 2

Recommendation: Minor Revision

Comments:
Still, the manuscript is not well prepared and needs to be improved.

Suggested improvements were not implemented. Alternatively, authors could explain reasons for not implementing these modifications.

Experimental procedure (cytotoxicity detection) is limited only to MTT method, and at least one additional confirmatory method is necessary. (Also, deeper insides in mechanisms of cytotoxicity could be implemented in this study.) Within experiments only three parallel measurements were conducted, for more reliable statistical analysis more data is needed.

English language needs improvement.

The authors should implement the above suggestions in their study or provide appropriate justification..

Additional Questions:
Does the manuscript contain new and significant information to justify publication?: No

Does the Abstract (Summary) clearly and accurately describe the content of the article?: Yes

Is the problem significant and concisely stated?: No

Are the methods described comprehensively?: Yes

Are the interpretations and conclusions justified by the results?: Yes

Is adequate reference made to other work in the field?: Yes

Length of article is: Adequate

Number of tables is: Adequate

Number of figures is: Adequate

Please state any conflict(s) of interest that you have in relation to the review of this paper (state "none" if this is not applicable).: None

Rating:

Interest: 3. Average

Quality: 4. Below Average

Originality: 3. Average

Overall: 3. Average

Reviewer: 3

Recommendation: Minor Revision

Comments:
May 22, 2020
Brazilian Research in Pediatric Dentistry and Integrated Clinic

Ref: Manuscript ID # PBOCI-2020-0048.R1

Title: "Lipopolysaccharide's Cytotoxicity on Human Umbilical Cord Mesenchymal Stem Cells"

The submitted manuscript was aimed to investigate the cytotoxicity of lipopolysaccharide (LPS) related to Porphyromonas gingivalis on human umbilical cord mesenchymal stem cells. The work is interesting and may contribute to the regenerative endodontics field. The in vitro experimental results seem to be interesting in future in vivo studies. However, the manuscript needs a minor revision before it is suitable for publication in this journal. Some comments are listed in the following.

The Introduction section needs to be improved. It remains unclear why it is important to study the relationship between LPS and HUCMSCs for dentistry applications. The reader cannot understand what these mean without reading the entire text of the manuscript. Please, provide information about it.

(Line 40, page 1) The authors report that: " One of the most common causes of tooth loss and endodontic treatment failures is periodontal disease by bacterial infection", in addition to endodontic and periodontal diseases, peri-implantitis infections should be added.

(Line 57, page 1) "Stem cells viability and response may differ depending on the time variation of contact from available LPS", give me more details about this sentence presenting previous works.

LPS preparation and cell model need to be more explained. References to specific methods can be included to make it more clear to the reader.

What statistical post-hoc test was used to investigate inter-group differences? This needs also to be

addressed.

In results section, the topic "Isolation and identification of HUCMSCs" should be only included in the materials and methods section and more explanations are needed about the sources of the antibodies.

The main results about the mean of cell viability (Table 1) and their microscopic images (Figure 1) not are explained in terms of cell distribution and morphology.

(Line 44, page 2) “The assessments were conducted in triplicates of n=3.”, the number of repeat experiments should be added.

3) Is it possible to estimate (quantification) the number of cells viable in each different LPS concentration exposed?

(Line 43, page 4) "LPS could enhance paracrine protective effects and regenerative healing of HUCMSCs". Please, explain better this sentence.

Also, even though the focus of this manuscript is on tissue regenerated, a major part of the success of the oral diseases and infection control is related to immune response, and the connection between cells and immune response should be addressed.

Lastly, limitations of the study and future perspectives were not stated.

Minor comments
(Line 54, page 1) "In-vitro" should be in vitro (in italic) but work through the entire manuscript carefully from this perspective.

Figure 1. Scale and magnification of the images are needed.

Table 2 is confusing and not needed. No difference was found between groups, thus this informations could be only included in the text.

Additional Questions:
Does the manuscript contain new and significant information to justify publication?: Yes

Does the Abstract (Summary) clearly and accurately describe the content of the article?: Yes

Is the problem significant and concisely stated?: No

Are the methods described comprehensively?: No

Are the interpretations and conclusions justified by the results?: Yes

Is adequate reference made to other work in the field?: No

Length of article is: Too short

Number of tables is: Too few

Number of figures is: Too few

Please state any conflict(s) of interest that you have in relation to the review of this paper (state “none” if this is not applicable).: None

Rating:

Interest: 2. Good

Quality: 3. Average

Originality: 2. Good

Overall: 3. Average

Date Sent: 23-May-2020

File 1: - Comments.pdf

File 2: - Review_PBOCI.pdf

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Decision Letter (PBOCI-2020-0048.R2)

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To: michael-j-k-k@fkg.unair.ac.id

CC:

Subject: Pesquisa Brasileira em Odontopediatria e Clínica Integrada - Decision on Manuscript ID PBOCI-2020-0048.R2

Body: 28-May-2020

Dear Dr. Kamadjaja:

It is a pleasure to accept your manuscript entitled "Lipopolysaccharide's Cytotoxicity on Human Umbilical Cord Mesenchymal Stem Cells" in its current form for publication in the Pesquisa Brasileira em Odontopediatria e Clínica Integrada.

Thank you for your fine contribution. On behalf of the Editors of the Pesquisa Brasileira em Odontopediatria e Clínica Integrada, we look forward to your continued contributions to the Journal.

Sincerely,
Prof. Alessandro Cavalcanti
Editor-in-Chief, Pesquisa Brasileira em Odontopediatria e Clínica Integrada
alessandrouepb@gmail.com

Date Sent: 28-May-2020

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Lipopolysaccharide's Cytotoxicity on Human Umbilical Cord Mesenchymal Stem Cells

By (Authors):
Mefina Kuntjoro, Eric Priyo Prasetyo, Febriastuti Cahyani, Michael Josef Kridanto Kamadjaja, Nike Hendrijantini, Harry Laksono, Primanda Nur Rahmania, Vivin Ariestania, Alexander Patera Nugraha, Igo Syaiful Ihsan, Aristika Dinaryanti, Fedik Abdul Rantam

Manuscript ID: PBOCI-2020-0048.R2

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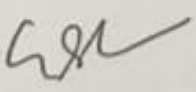
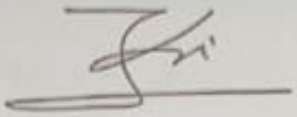
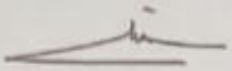
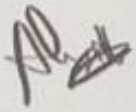
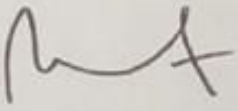
	NAME	SIGNATURE	DATE
1	Mefina Kuntjoro		2 August 2020
2	Eric Priyo Prasetyo		2 August 2020

3	Febriastuti Cahyani		2 August 2020
4	Michael Josef Kridanto Kamadjaja		2 August 2020
5	Nike Hendrijantini		2 August 2020
6	Harry Laksono		2 August 2020
7	Primanda Nur Rahmania		2 August 2020
8	Vivin Ariestania		2 August 2020
9	Alexander Patera Nugraha		2 August 2020
10	Igo Syaiful Ihsan		2 August 2020
11	Aristika Dinaryanti		2 August 2020
12	Fedik Abdul Rantam		2 August 2020

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ACTION	STATUS	ID	TITLE	SUBMITTED	DECISIONED
	ADM: Valença, Ana Maria • Accept (28-May-2020) *Archiving completed on 26-Nov-2020* view decision letter Contact Journal	PBOCI-2020-0048.R2	Lipopolysaccharide's Cytotoxicity on Human Umbilical Cord Mesenchymal Stem Cells *Files Archived*	26-May-2020	28-May-2020

ACTION	STATUS	ID	TITLE	SUBMITTED	DECISIONED
a revision has been submitted (PBOCI-2020-0048.R2)	ADM: Valença, Ana Maria • Minor Revision (23-May-2020) • a revision has been submitted *Archiving completed on 26-Nov-2020* view decision letter Contact Journal	PBOCI-2020-0048.R1	Lipopolysaccharide's Cytotoxicity on Human Umbilical Cord Mesenchymal Stem Cells *Files Archived*	28-Apr-2020	23-May-2020
a revision has been submitted (PBOCI-2020-0048.R1)	ADM: Valença, Ana Maria • Major Revision (23-Apr-2020) • a revision has been submitted *Archiving completed on 26-Nov-2020* view decision letter Contact Journal	PBOCI-2020-0048	Lipopolysaccharide's Cytotoxicity on Human Umbilical Cord Mesenchymal Stem Cells *Files Archived*	10-Mar-2020	23-Apr-2020

Pesquisa Brasileira em Odontopediatria e Clínica Integrada 2020; 20:e0048
https://doi.org/10.1590/pboci.2020.155
ISSN 1519-0501 / eISSN 1983-4632

ORIGINAL ARTICLE

Lipopolysaccharide's Cytotoxicity on Human Umbilical Cord Mesenchymal Stem Cells

Mefina Kuntjoro[1,2], Eric Priyo Prasetyo[3], Febriastuti Cahyani[3], Michael Josef Kridanto Kamadjaja[2], Nike Hendrijantini[2], Harry Laksono[2], Primanda Nur Rahmania[2], Vivin Ariestania[4], Alexander Patera Nugraha[1,5], Igo Syaiful Ihsan[6], Aristika Dinaryanti[6], Fedik Abdul Rantam[6,7]

[1]Doctoral Program, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.
[2]Department of Prosthodontics, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
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Author to whom correspondence should be addressed: Michael Josef Kridanto Kamadjaja, Department of Prosthodontics, Faculty of Dental Medicine, Universitas Airlangga, Jalan Mayjen Prof Dr Moestopo 47, Surabaya, Indonesia. Phone: +62 315030255. E-mail: Michael-j-k-k@fkg.unair.ac.id.

Academic Editor: Yuri Wanderley Cavalcanti

Received: 10 March 2020 / Accepted: 28 May 2020 / Published: XX August 2020

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Abstract

Objective: To show the cytotoxicity of *Porphyromonas gingivalis* lipopolysaccharide (LPS) on human umbilical cord mesenchymal stem cells (HUCMSCs) to better understand the characteristics for its application in regenerative procedures under periodontopathogen LPS influence. **Material and Methods:** Ultrapure *Porphyromonas gingivalis* LPS was used in this study. This research used a frozen stock HUCMSCs, previously confirmed by flow cytometry. The biological characteristics, such as cell morphology, proliferation, and protein expression, were screened. To check the cytotoxicity, HUCMSCs were cultured and divided into two groups, the control group and LPS group with various concentrations from 25 to 0.39 µg/mL. MTT assay was done and the cells were observed and counted. The significance level was set at 5%. **Results:** The percentage of living HUCMSCs on LPS group were not significantly different among concentrations ($p>0.05$) from 25 to 0.39 µg/mL, even though there were slight mean decrease between groups, but they were not significant. The duration of 24 hours of exposure of LPS does not significantly lower HUCMSCs viability. **Conclusion:** LPS does not affect the viability of HUCMSCs. The lower the concentration of LPS, the higher the viability of HUCMSCs.

Keywords: Lipopolysaccharide; Viability; Umbilical cord; Mesenchymal stem cells; Cytotoxicity.

Introduction

There are several mesenchymal stem cells (MSCs) sources available for clinical applications. Human umbilical cord mesenchymal stem cells (HUCMSCs) are MSCs found in the umbilical cords and may be a prospective candidate for use in tissue regeneration [1]. Compared to other sources of stem cells from adult tissues, HUCMSCs are more primitive, non-invasive in terms of collection, provide high proliferation potential, high differentiation potential, immune-privileged, immunosuppressive, and rich in stemness [2].

One of the most common causes of tooth loss and endodontic treatment failures is a periodontal disease by a bacterial infection. Many of the infection is caused by gram-negative bacilli, such as *Porphyromonas gingivalis* [3,4]. These bacteria also cause periodontitis, peri-implantitis, and peri-implant mucositis infections [5,6]. Lipopolysaccharide (LPS) is an outer membrane unit of Gram-negative bacterial cell walls. It is also an endotoxin with a wide range of biological maneuvers. LPS can induce the release of several pro-inflammatory cytokines, which may lead to alveolar bone resorption and periodontal tissue destruction [7].

LPS does not indicate a single molecular type, but a molecule of diverse chain lengths with high and low molecular weights, which affect the biological activity [8]. Several studies have evaluated the increase of functional properties of MSCs by LPS exposure to defend against a harsh inflammatory environment [9,10]. However, few studies have used HUCMSCs on LPS exposure. Cell cultures are a useful technique to evaluate the biocompatibility of various materials [11,12]. *In-vitro* assessment of cell cultures are usually used to explore the mechanism and biological responses in certain conditions.

Even though results from *in-vitro* experiments cannot immediately be generalized to mimic clinical conditions, they are relevant as they reflect a model for screening of various material properties and risks [13]. By studying the viability of HUCMSCs under *Porphyromonas gingivalis* LPS influence, we can study further approach of regenerative procedures under this influence, such as in endodontics, periodontics, prosthodontics (implants) failures affected by periodontopathogen LPS. Previous studies about LPS on other stem cell sources, such as human periodontal ligament stem cells and stem cells from the apical papilla have used *Porphyromonas gingivalis* LPS but in limited concentrations, ranging from 0 to 5 μg/mL or 0 to 10 μg/mL, with different and irregular intervals [14,15].

Stem cells' viability and response may differ depending on the variation of contact from available LPS. An in-vitro study of LPS exposure on the apical papilla's stem cells did not affect its proliferation and mineralization. At a concentration of 5 mg/mL, LPS increased bone sialoprotein gene expression [14].

There are other studies with non-periodontopathogen LPS from *E. coli* showed different results on how LPS effect stem cells in terms of reduced proliferation and growth factor secretion [16]. Therefore, as the first step of understanding HUCMSCs under periodontopathogen LPS condition, the purpose of this study was to investigate the cytotoxicity of various concentrations of *Porphyromonas gingivalis* LPS on the viability HUCMSCs, through MTT assay.

Material and Methods

Ethical Aspects

This study was permitted by the Health Research Ethical Clearance Commission, Universitas Airlangga Faculty of Dental Medicine, Surabaya, Indonesia (Clearance number 060/HRECC.FODM/II/2020).

Preparation of HUCMSCs

Previously isolated and characterized frozen stock HUCMSCs from the 4th passage was obtained from Stem Cell Research and Development Center Universitas Airlangga (Surabaya, Indonesia). The HUCMSCs

was previously confirmed by flow cytometric analysis using FACS Calibur (BD Biosciences, USA) and identified by specific antibodies for CD73, CD90, CD105, CD45, and CD34. There were positivity of CD73, CD90, CD105, negativity of CD45 and CD34. All antibodies were purchased from Becton-Dickinson (BD Biosciences, San Jose, CA USA).

The frozen HUCMSCs from -80°C storage were thawed in a water bath with a temperature of 37°C. Thawed HUCMSCs were moved to another tube containing 10 mL Minimum Essential Medium (MEM) alpha medium (Gibco, Paisley, UK) at 37°C, then centrifuged for 5 minutes at 1600 rpm.

The supernatant was eliminated and the cell pellet was resuspended in culture medium in a 12 mm diameter plate. The resuspended cells were stored in an incubator for 6 hours at 37°C. The evaluation was done using TMS inverted microscope (Nikon, Minato, Japan). The thawing of HUCMSCs was successful; the cells were expanded and ready for further treatment.

Lipopolysaccharide (LPS) Preparation for HUCMSCs

Ultrapure *Porphyromonas gingivalis* lipopolysaccharide (InvivoGen, San Diego, CA, USA) was used [17]. The LPS powder was diluted with MEM alpha medium into different concentrations. LPS dilution was done following the manufacturer's directions. LPS concentrations of 0.39, 0.78, 1.56, 31.25, 6.25, 12.5, and 25 µg/mL were to be compared in this experiment. This concentration was observed because numerous studies are using different concentrations; therefore, we decided to divide and range it from 25 to 0 µg/mL gradually in a regular pattern. The experiments were performed in triplicate.

Assessment of Viable HUCMSCs

The viability of HUCMSCs on LPS was determined by methyl-thiazol-tetrazolium (MTT). The fifth passage HUCMSCs were seeded at a density of 5,000 cells per well in a 96-well culture plate (Iwaki, Asahi, Japan) and treated with LPS at concentrations ranging from 0.05-25 µg/mL for 24 hours. Each well was given 200 microliter MEM alpha medium containing LPS according to the explored concentrations.

After incubation, MTT was added to each well and incubated for 3 hours, and then the process was stopped by the addition of 50 microliters of DMSO per well. Cell proliferation was examined by measuring optical density from a microplate reader (GloMax®Explorer, Promega Corporation, Fitchburg, WI, USA) at a wavelength of 595 nm. Viable cells were seen under an inverted TMS microscope (Nikon Corp., Tokyo, Japan) with 100x magnification for cell distribution and morphology among the control and observed groups.

Statistical Analysis

The assessments were conducted and performed in triplicates with regard to previous studies [16,17] and sample size determination formula for controlled laboratory experiments. Data were provided as mean + standard deviation. Statistical analysis was done using SPSS20.0 for Windows (SPSS Inc., Chicago, Illinois, USA). All data were tested for normal distribution. One-way ANOVA test was used to compare 3 or more groups (among control and LPS groups of different concentrations). Post Hoc LSD (Least Significant Difference) test was used to investigate multiple comparisons of inter-group differences. The difference between groups was considered significant when $p<0.05$.

Results

Viable cells seen under an inverted microscope with 100x magnification are available in Figure 1. There was no difference in cell distribution and morphology among the control and observed groups. The mean and standard deviation (SD) of control and lipopolysaccharide (LPS) groups are shown in Table 1.

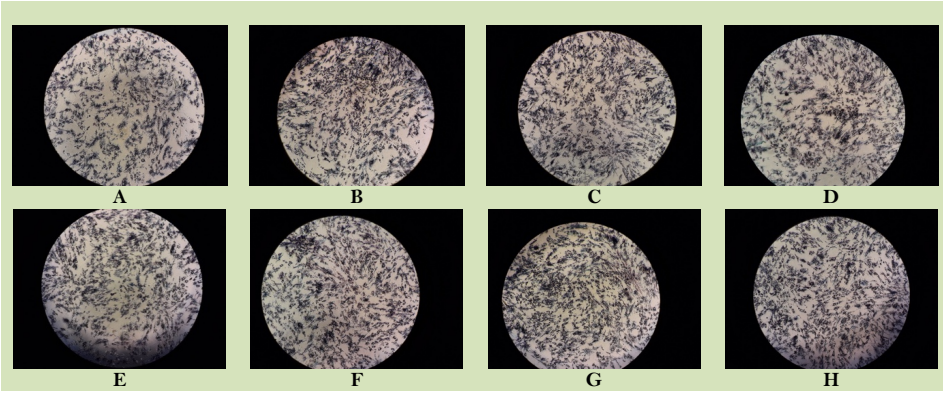

Figure 1. Viable cells seen under inverted microscope (100x magnification): control (A), LPS concentration of 25 µg/mL (B), 12.5 µg/mL (C), 6.26 µg/mL (D), 3.125 µg/mL (E), 1.56 µg/mL (F), 0.78 µg/mL (G), and 0.39 µg/mL (H).

Data in the result was normally distributed ($p>0.05$), and homogeneity test yield homogeny data ($p>0.05$). MTT assay result of different lipopolysaccharide (LPS) concentrations showed no significant difference among 0.39, 0.78, 1.56, 3.12, 5.625, 12.5 and 25 µg/mL ($p>0.05$).

Table 1. Mean and standard deviation of control and LPS groups.

Group	ercentage of Cell Viability Mean (SD)
Control	100 ± 0.00
LPS 25	69.8193 ± 4.02339
LPS 12.5	66.4653 ± 4.22801
LPS 6.25	68.3773 ± 3.00004
LPS 3.125	68.7017 ± 2.88213
LPS 1.56	67.1853 ± 3.32384
LPS 0.78	66.9630 ± 2.51683
LPS 0.39	67.1697 ± 2.91831

Discussion

There is a higher interest in searching the prospects of human umbilical cord mesenchymal stem cells (HUCMSC) because this stem cell can be isolated and expanded easily in large quantities, and non-invasive compared to commonly used bone marrow mesenchymal stem cells [18]. There are studies about other MSC sources, but little information was available regarding HUCMSC and its potential use in pulpal and periapical regeneration.

Pulpal and periapical regenerative researches using MSCs are hoped to be an alternative to treat various pathological conditions, such as chronic inflammation involving lipopolysaccharide (LPS). However, it stays unclear how LPS could influence MSC to resolve inflammation. Some research on LPS preconditioning of MSC has been an increasing approach for tissue injury and inflammatory disease therapy [19].

HUCMSCs were cultured in minimum essential medium alpha (MEM-A) containing ultrapure *Porphyromonas gingivalis* LPS in various concentrations from 0 to 25 µg/mL. We chose this range of concentrations because previous studies use different and limited concentrations of LPS in their methods; therefore, we expand the concentrations from 0 to 25 µg/mL. We use LPS from *Porphyromonas gingivalis*

because it is among the most common species in primary root canal infection [14]. The cells were incubated under normal culture conditions at 37°C and 5% CO_2.

We used MTT assay to determine cell viability and determine cytotoxicity under periodontopathogen LPS influence. MTT assay is mostly chosen to determine cytotoxicity and proven to be more accurate and practical. The MTT assay principle is to break the tetrazolium ring (3-(4,5-dimethylthiazole-2-yl)-2,5 diphenyl tetrazolium bromide) to produce insoluble blue-purple formazan crystals. The living cells can be measured using optical density spectrophotometry. The lower the optical density means the lower living cells to metabolize MTT.

In this study, there were no differences in HUCMSCs viability among various concentrations of LPS, meaning LPS is not toxic to HUCMSCs within these concentrations. This finding might assume that LPS could enhance paracrine protective effects and regenerative healing of HUCMSCs. This condition might be related with the finding of other studies that LPS influence mesenchymal stem cells to create a suitable milieu to promote tissue repair and reduce inflammation through a paracrine mechanism and exosomes [20].

Exosomes are small vesicles carrying bioactive molecules, such as microRNAs (miRNAs), messenger RNAs (mRNAs) and protein. These bioactive molecules can be passed between cells to influence cellular activities and metabolism in recipient cells [21,22]. Exosomes contain many genetic molecules that act as cell-to-cell communication channels to alter genetic expression in recipient cells [19].

Previous study on human periodontal ligament cells showed that both pretreatment and non-pretreatment of LPS have higher production of pro-inflammatory mediators [23]. LPS plays a crucial role in the pathogenesis of apical lesions by releasing pro-inflammatory cytokines. We found that LPS has no toxicity on HUCMSCs on the given concentrations. This research is in line with previous study on stem cells from the apical papilla that LPS concentration of 0 to 5 µg/mL did not affect cell viability [14]. This might be caused by the fact that LPS have induced HUCMSCs to hold a cytoprotection potential, but further study needs to be done. The survival of HUCMSCs will ensure the paracrine effect for tissue regeneration and regulation of anti-inflammation through an immunomodulatory effect on immune cells responses [24,25].

Conclusion

These results show LPS does not significantly affect the viability of HUCMSCs depending on the concentration used. In conclusion, this study provides evidence that LPS is not toxic to HUCMSCs within tested concentrations. However, this study is limited to *Porphyromonas gingivalis* LPS within a range of 0 to 25 µg/mL, to find out more, further studies need to be conducted with higher concentrations. Further *in-vitro* and *in-vivo* studies will also be required to reveal more novel mechanisms of *Porphyromonas gingivalis* LPS on HUCMSCs for regenerative purposes in endodontics, periodontics, prosthodontics, pediatric dentistry, oral and maxillofacial surgery.

Authors' Contributions

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ISI	0000-0001-9038-0358	Methodology, Software, Validation, Formal Analysis, Investigation and Data Curation.
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FAR	0000-0001-8182-1465	Methodology, Investigation, Resources, Supervision and Project administration.

All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

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