



Meircurius Dwi Condro Surboyo <meircurius-2015@fkg.unair.ac.id>

EJD-2019-12-18 - (328) Revise manuscript (minor revisions)

1 pesan

European Journal of Dentistry <ejd@manuscriptmanager.net>

3 Januari 2020 05.15

Balas Ke: Nejdeth Adanir <necdethan@gmail.com>

Kepada: meircurius-2015@fkg.unair.ac.id

Manuscript: EJD-2019-12-18 - (328) - Concanavalin A Enhanced Proliferation and Osteogenic Differentiation of Dental Pulp Stem Cells

Date submitted: 2019-12-08

Dear Dr. Surboyo

Thank you very much for submitting the above manuscript to European Journal of Dentistry.

Your manuscript has now been evaluated by external reviewers and members of the editorial board. A number of minor points of criticism were raised and these must be addressed before your manuscript can be accepted for publication.

When you resubmit your manuscript, please provide point-by-point answers to the reviewer criticisms in the text field provided during the online resubmission process and highlight any revisions made in your manuscript document.

We look forward to receiving the revised version and thank you, again, for submitting your manuscript to European Journal of Dentistry.

Sincerely,

Dr. Nejdeth Adanir, DDS, PhD.
Editor-in-Chief, European Journal of Dentistry
Associate Professor, Department of Restorative Dentistry
School of Dentistry, King Faisal University
Al Ahsa, KSA, 31982
necdethan@gmail.com

Reviewer report:

the following sentences need to be rephrased (correct English) to make the meaning clear for the audience:

In the Introduction section :

- Paragraph 1 line 6: "The ability to differentiate into odontoblasts cells causing DPSCs can be used as a component for regenerative dentine formation indirect pulp capping therapy."

- Paragraph 3 line 3: Plant lectins have a role cellular process

In the Results section: 1st & 2nd paragraph

A concentration of 10 µg/ml produced the highest proliferation and osteogenic differentiation ability. Since the paper compares only two concentrations (5 and 10 µg/ml), results should be rephrased to A concentration of 10 µg/ml produced a higher proliferation and osteogenic differentiation ability than 5 µg/ml.



Meircurius Dwi Condro Surboyo <meircurius-2015@fkg.unair.ac.id>

EJD-2019-12-18/R1 RESUBMISSION - (328) Accept manuscript

1 pesan

European Journal of Dentistry <ejd@manuscriptmanager.net>

8 Januari 2020 17.24

Balas Ke: Nejdeth Adanir <necdethan@gmail.com>

Kepada: meircurius-2015@fkg.unair.ac.id

Manuscript: EJD-2019-12-18/R1 RESUBMISSION - (328) - Concanavalin A Enhanced Proliferation and Osteogenic Differentiation of Dental Pulp Stem Cells

Date submitted: 2020-01-06

Dear Dr. Surboyo

It is a pleasure to inform you that your manuscript is now acceptable for publication in European Journal of Dentistry. Proofs of your manuscript, Copyright Transfer agreement, and details for making the APC payment will be sent by the production team in due course.

Thank you,

Sincerely,

Dr. Nejdeth Adanir, DDS, PhD.
Editor-in-Chief, European Journal of Dentistry
Associate Professor, Department of Restorative Dentistry
School of Dentistry, King Faisal University
Al Ahsa, KSA, 31982
necdethan@gmail.com

YOUR SIGN IN INFORMATION

Website: <https://www.manuscriptmanager.net/ejd>

Email: meircurius-2015@fkg.unair.ac.id

Forgot password or not signed in before?

Click the URL below to create/reset your password.

https://www.manuscriptmanager.net/sLib/v4/retrieve_pw.php?paramScreen=3N+y+zm+kQ2DMYBp5F7sYld59WGGdcL+8T8C10Wg1lo=

Editor-in-Chief comments:

Cross-referencing: We would like to emphasize that we attach great importance to cross-referencing recent papers on the same topic in the European Journal of Dentistry. Therefore, it would be highly appreciated if you would check the last 5 years of European Journal of Dentistry and cite references relevant to your article.

<https://www.ncbi.nlm.nih.gov/pubmed/?term=eur+j+dent>

YOUR SIGN IN INFORMATION

Website: <https://www.manuscriptmanager.net/ejd>

Email: meircurius-2015@fkg.unair.ac.id

Forgot password or not signed in before?

Click the URL below to create/reset your password.

https://www.manuscriptmanager.net/sLib/v4/retrieve_pw.php?paramScreen=Scfo1Ci+W/P6dQbme/50y3ep6fkvt47HEoEaD9qilGE=



udijanto tedjosasongko <udijanto@fkg.unair.ac.id>

RE: Final proof

2 messages

Manpreet Kaur <manpreet.kaur@thieme.in>
To: Meircurius Dwi Condro Surboyo <meircurius-2015@fkg.unair.ac.id>
Cc: udijanto@fkg.unair.ac.id

Wed, Feb 5, 2020 at 7:45 PM

Dear Dr. Subroyo,

The proofs of the article were released on Feb 3 to the corresponding author. Please find attached the same.

Thank you.

Regards,

Manpreet

Dr. Manpreet Kaur
Project Manager -Journals
Thieme Medical and Scientific Publishers Private Limited
A-12, Second Floor, Sector -2,
NOIDA -201301
Uttar Pradesh
India

Email: manpreet.kaur@thieme.in

Tel: +91 120 4556645

Fax: +91 120 4556649

www.thieme.com

Visit us at www.thieme.com; www.thieme.in

From: Meircurius Dwi Condro Surboyo <meircurius-2015@fkg.unair.ac.id>
Sent: Wednesday, February 05, 2020 5:34 PM
To: Manpreet Kaur <manpreet.kaur@thieme.in>
Subject: Final proof

Dear Manpreet Kaur

I need information related my articles, when the final proof release to us?

—

Manuscript ID: **EJD-2019-12-18/R1 RESUBMISSION - (328)** Meircurius Dwi Condro Surboyo, drg.,M.Kes
Lecturer

Concanavalin A Enhanced Proliferation and Osteogenic Differentiation of Dental Pulp Stem Cells

Regarrd
Department of Oral Medicine
Faculty of Dental Medicine
Universitas Airlangga

+6287854376754

meircurius@fkg.unair.ac.id; meircurius@gmail.com

 **EJD-19-00328.pdf**
3363K

udijanto tedjosasongko <udijanto@fkg.unair.ac.id>
To: suardita@hotmail.com

Wed, Feb 5, 2020 at 8:13 PM

----- Forwarded message -----

From: Manpreet Kaur <manpreet.kaur@thieme.in>
Date: Wed, Feb 5, 2020, 7:46 PM
Subject: RE: Final proof
To: Meircurius Dwi Condro Surboyo <meircurius-2015@fkg.unair.ac.id>
Cc: <udijanto@fkg.unair.ac.id>

Dear Dr. Subroyo,

The proofs of the article were released on Feb 3 to the corresponding author. Please find attached the same.

Thank you.

Regards,

Manpreet

Dr. Manpreet Kaur

Project Manager -Journals

Thieme Medical and Scientific Publishers Private Limited

A-12, Second Floor, Sector -2,

NOIDA -201301

Uttar Pradesh

India

Email: manpreet.kaur@thieme.in

Tel: +91 120 4556645

Fax: +91 120 4556649

www.thieme.com

Visit us at www.thieme.com; www.thieme.in

From: Meircurius Dwi Condro Surboyo <meircurius-2015@fkg.unair.ac.id>

Sent: Wednesday, February 05, 2020 5:34 PM

To: Manpreet Kaur <manpreet.kaur@thieme.in>

Subject: Final proof

Dear Manpreet Kaur

I need information related my articles, when the final proof release to us?

--

Manuscript ID: **EJD-2019-12-18/R1 RESUBMISSION - (328)**

Meircurius Dwi Condro Surboyo, drg.,M.Kes

Lecturer

Concanavalin A Enhanced Proliferation and Osteogenic Differentiation of Dental Pulp Stem Cells

Regarrd

Department of Oral Medicine

Faculty of Dental Medicine

Universitas Airlangga

+6287854376754

meircurius@fkg.unair.ac.id; meircurius@gmail.com



EJD-19-00328.pdf
3363K

Instructions to Contributors

Dear Contributor:

Enclosed in this document please find the page proofs and copyright transfer agreement (CTA) for your article in the *European Journal of Dentistry*. Please print this document and complete and return the CTA along with corrected proofs, within 72 hours.

- 1) Please read proofs carefully for **typographical** and **factual** errors only; mark corrections in the margins of the proofs in blue or black pen, or use **Adobe Acrobat tools** to mark the changes in the PDF file directly. Please be sure to write as clearly as possible so no errors are introduced into your article. **Answer (on the proofs) all author queries marked in the margins of the proofs.** Check references for accuracy. **Please check on the 1st page of your article that your titles and affiliations are correct.** Avoid elective changes, because these are costly and time consuming and will be made at the publisher's discretion.
- 2) Please pay particular attention to the proper placement of figures, tables, and legends. Please provide copies of any formal letters of permission that you have obtained.
- 3) As a contributor to this journal you will receive a complimentary PDF file of the article after publication.
 - If you wish to order offprints, **please circle the quantity required** (left column) **and the number of pages in your article.** If you wish to order copies of the journal please enter the number of copies on the indicated line.
 - If you do not want to order offprints or journals simply put a slash through the form, **but please return the form.**

Please return all materials within 72 hours. E-mail is the easiest way to ensure your corrections are received in a timely manner. Please return the corrected proofs to:

Manpreet Kaur, Project Manager
Thieme Medical and Scientific Publishers
Fax: +91-120-4556649
E-mail: : manpreet.kaur@thieme.in

Please do not return your materials to the editor or the typesetter.

Please note: Due to a tight schedule, if the publisher does not receive the return of your article proofs within 7 days of the date the e-mail was sent to you, the publisher reserves the right to proceed with publication without author changes. Such proofs will be proofread by the editor and the publisher.

Thank you for your contribution to this journal.

Permission to Publish and Open Access Copyright Transfer Agreement

Manuscript Information:

Journal: European Journal of Dentistry

Manuscript Title: Concanavalin A Enhanced Proliferation and Osteogenic Differentiation of Dental Pulp Stem Cells

Manuscript Number: EJD-19-00328

Authors: Ketut Suardita, Ira Arundina, Udijanto Tedjosongko, Anita Yuliati, Harry Huiz Peeters, I Komang Evan Wijaksana, Meircurius Dwi Condro

Corresponding author's contact data: Udijanto Tedjosongko , Departmen of Pediatric Dentistry, Faculty of Dental Medicine, Universitas Airlangga. Jalan Prof. Dr. Moestopo 47 Surabaya, 60132, Indonesia


Corresponding author's e-mail address: udijanto@fkg.unair.ac.id

Contact at the publishers: Manpreet Kaur

E-mail address at the publishers: manpreet.kaur@thieme.in

Dear Author,

Please

- read this form carefully,
- check all manuscript information,
- sign this form with your digital signature and
- return to us by clicking on 

Thank you very much in advance.

Assignment of Rights

The Thieme Publishing Group does not accept for publication in a journal any manuscript that has been published elsewhere. Your consent to the following assignments of rights, also on behalf of the other authors (if several authors contribute to the manuscript), and the signing of this Open Access Copyright Transfer Agreement is a necessary requirement for the publication of your manuscript.

Without prejudice to this assignment of rights, the moral rights in connection with the article shall remain with you and the other authors.

The article will be published under a CC-BY-NC-ND license (<https://creativecommons.org>). This license means that anyone may freely read, download, distribute and make the article available to the public (in printed and electronic form), provided that the author and the journal as the source are acknowledged, whereas no commercial use is allowed and the work may not be altered, transformed or serve as the basis for a derivative work.



Thieme will endeavor to ensure maximum dissemination of your manuscript and to protect your authors' rights against misuse, such as plagiarism.

For this reason upon acceptance of the manuscript by us you and your co-authors agree to hereby transfer on a non-exclusive basis copyright to Thieme. This includes (in addition to the publication under a CC-BY-NC-ND license), for the duration of the statutory term of copyright protection and throughout the world, the full rights to use the paper (including excerpts thereof) within the journal or separate to it, on a commercial or non-commercial basis, linked to other works or papers, audio-visual accompanying materials or interactive products or services, including the transfer to third parties (e.g., under license agreements, etc.), in particular the non-exclusive right to use all editions/updates for the following purposes:

– reproduction and distribution in printed form, in particular as a journal article, article in a medical textbook or other type of book directed towards a specific target group of readers, pocket book, special edition for secondary markets or special customers, brochure, supplement, anthology, etc.;

Please use the latest Adobe Reader version independently of your system software (Windows, Linux, Mac OS X).
To download Adobe Reader: [click here](#).

Permission to Publish and Open Access Copyright Transfer Agreement

— reproduction and distribution in the form of electronic media (e.g., magnetic tape, CD-ROM, CDI, DVD, electronic paper or chips, e-book, RAM, hard disk, USB-based storage media, etc.), making available to the public (e.g., the Internet, intranets or other wired or wireless data networks), in particular by displaying such data on stationary or mobile visual display units, screens, personal digital assistants, mobile phones, smart phones or other receiver terminals by download (e.g., as an epub, app or PDF file) or retrieval in any other form;

— translation, transmission or adaptation of the paper into or in any other language, dialects or versions (in particular, as a podcast, audio book, or other image or sound carriers etc.), transmission through television, cable or satellite TV, radio or by means of other audio-visual media, renting or lending, storage in an electronic archive, including storage in connection with indexation, key word search or any other search and additional functions, usage for types of use not as yet known and for any other rights used by collecting societies as provided in their articles and allocation plans, provided that the transfer of such rights is permissible under the relevant provisions and applicable law; you also assign to Thieme all statutory royalty claims under §§ 44a et seq. Urheberrechtsgesetz (German Copyright Law) insofar Thieme mandates a collecting society to administer such rights jointly for authors and publishers.

Thieme is entitled, but not obliged, to use the rights specified in the aforesaid sections and may adapt the article for these uses. Thieme will take your and your co-authors' legitimate interests into account in this respect.

Authors' rights and Gold Open Access

The rights of use are assigned to us non-exclusively – subject to your rights in accordance with our Open Access (OA) Guidelines: Our OA Guidelines state that immediately after the publication of the article by us, you and the other authors are entitled to make the published version of the article available to the public. For further details please click the button "Authors' rights and Gold Open Access."



Authors' rights and Gold Open Access

For more Information on our OA Program please visit <http://open.thieme.com>.

Article Publication Charge

Publication of the article is subject to an article publication charge of (incl. VAT if any).
The article will be published upon receipt of the payment by Thieme.

Duties of care

Product liability laws set high standards for your duty of care as the author of a scientific manuscript. This is especially the case when you give therapeutic information and/or specify drug doses. Therefore please check this information carefully in the typeset page-proofs of your article. Your task will be much easier if you have the information counterchecked – depending on the sensitivity of the information within the article – by specialist colleagues. Only you, as the author, have the specialist knowledge to be able to assess the accuracy of the information. For further information on how to indicate corrections, please click the button "Correction markup symbols."



Correction markup symbols

Author's Declaration

I have taken note of the information on the duties of care under product liability law; I agree to the assignments of rights in accordance with the foregoing sections "Assignment of Rights" and "Authors' Rights and Gold Open Access" also on behalf of the other authors (if several authors have contributed to the article). I declare that I am authorized by my co-authors to sign on their behalf.

I declare that no third party rights will be infringed through the publication. Any material contained in the manuscript (including illustrations, tables, or other material) from third-party sources will be identified as such through citation, indicating the source. If required, I have obtained the copyright permission from the publishers concerned.

I have read and understood the terms and conditions of the CC-BY-NC-ND license and I agree also on behalf of the other authors (if several authors have contributed to the article) **that the article will be published under such a license.**

I agree with the aforesaid article publication charge. Should one of the foregoing regulations be or become invalid in whole or in part this shall not affect the validity of the other provisions. Any invalid provision shall be replaced by a regulation that comes as close as possible to the purpose of the invalid provision in economic terms, insofar as legally permissible.

This article is ready to be published after the execution of the corrections indicated by me.

Date

Digital Signature



Digital signature



Concanavalin A Enhanced Proliferation and Osteogenic Differentiation of Dental Pulp Stem Cells

Ketut Suardita¹ Ira Arundina² Udijanto Tedjosongko³ Anita Yulianti⁴ Harry Huiz Peeters⁵


Q1 I Komang Evan Wijaksana⁶ Meircurius Dwi Condro Surboyo⁷  Q1

¹Department of Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga, Jawa Timur, Indonesia

²Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Jawa Timur, Indonesia

³Department of Pediatric Dentistry, Faculty of Dental Medicine, Universitas Airlangga, Jawa Timur, Indonesia

⁴Department of Dental Material, Faculty of Dental Medicine, Universitas Airlangga, Jawa Timur, Indonesia

Q2 ⁵Laser Research Center in Dentistry, Bandung, Indonesia  Q2

⁶Department of Periodontics, Faculty of Dental Medicine, Universitas Airlangga, Jawa Timur, Indonesia

⁷Department of Oral Medicine, Faculty of Dental Medicine, Universitas Airlangga, Indonesia

Address for correspondence  Udijanto Tedjosongko, Department of Pediatric Dentistry, Faculty of Dental Medicine, Universitas Airlangga, Jalan Prof. Dr. Moestopo 47 Surabaya, 60132, Indonesia (e-mail: udijanto@fkg.unair.ac.id).

Q3

Eur J Dent 2020;00:1–5

Abstract

Objective Dental pulp stem cells (DPSCs) can be used as a component in the formation of regenerative dentine during direct pulp capping therapy. Concanavalin A (ConA) is a type of lectin with a molecular weight of 26 kDa derived from the *Canavalia ensiformis* plant. Lectins possess strong proliferation and differentiation abilities in various animal cells including lymphocytes, osteoblasts, and chondrocytes. The aim of study was to determine the effect of ConA on the proliferation and osteogenic differentiation of DPSCs *in vitro*.

Materials and Methods In this *in vitro* study, DPSCs were isolated from third molars before ConA induction was performed at concentrations of 5 and 10 µg/mL. The proliferation assay was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Osteogenic differentiation was determined by means of mineralization.

Statistical Analysis Data were analyzed using analysis of variance and a Student's *t*-test. The *p*-value was set at 0.05.

Results The addition of 5 and 10 µg/mL of ConA to DPSCs can significantly increase the proliferation and osteogenic differentiation of DPSCs (*p* ≤ 0.05).

Conclusion ConA can increase the proliferation and osteogenic differentiation of DPSCs.

Keywords

- ▶ concanavalin A
- ▶ dental pulp
- ▶ differentiation
- ▶ proliferation
- ▶ stem cell

Introduction

Dental pulp stem cell (DPSC) is one of the promising dental stem cells for regenerative therapy. DPSCs show a higher proliferation rate compared with bone marrow mesenchymal stem cells (MSCs). In addition, DPSCs also have a stronger ability to differentiate into osteoblasts; therefore, DPSCs more

appropriate for cell-based therapy.¹ DPSCs are multipotent and can differentiate into chondrocytes, adipocytes, osteoblasts/osteocytes, myocytes, nerve cells, cardiomyocytes, and odontoblasts.^{2–4} DPSCs can be used to regenerate periodontal and dental tissue because they have the potential to form tissues such as bone, and are able to form dentin and pulp.^{2,5–7} With the ability to differentiate into odontoblast, DPSCs can

be used as a component for regenerative dentine formation in direct pulp capping therapy.

The number of stem cells within the body is very limited. Consequently, the method of increasing their number in vitro without also affecting their differentiation must be developed. Several types of growth factors have been used to improve stem cell proliferation capability such as fibroblast growth factor-2 (FGF-2) or transforming growth factor- β 1 (TGF- β 1).⁸ In addition, various factors are used to induce the differentiation capability of MSCs, for example, by using recombinant bone morphogenic protein-2 (BMP-2), which can increase the osteogenic differentiation of MSC.⁹ However, safer, cheaper, and more effective growth factors have yet to be identified.

In this study, concanavalin A (ConA) was investigated as a novel factor that may enhance the proliferation and osteogenic differentiation of DPSCs. ConA is a type of lectin with a molecular weight of 26 kDa derived from the *Canavalia ensiformis* plant. Plant lectins play an important role in the cellular process. Lectins have strong proliferation and differentiation abilities in variety of animal cells, including lymphocytes, osteoblasts, and chondrocytes.^{10,11} The addition of ConA can enhance the process of mineralization or calcification of MSC derived from bone marrow. ConA can also increase levels of osteocalcin and BMP-2 proteins in MSC culture media.¹² However, the effects of ConA on the proliferation and differentiation of DPSCs have yet to be investigated. The aim of this study was to determine the effect of ConA on the proliferation and osteogenic differentiation of DPSCs in vitro.

Materials and Methods

DPSCs Isolation from the Pulp of Third Molars

Ethical clearance for the research was obtained from the Health Research Ethical Clearance Commission, Faculty of Dental Medicine Universitas Airlangga (approval number 13/KKEPK.FKG/1/2016). In this in vitro study, dental pulp was extracted from the impaction vital teeth of healthy adults. Following extraction, teeth were placed into sterile phosphate-buffered saline (PBS; Sigma-Aldrich, Missouri, United States) solution. The teeth were sectioned axially at the cemento-enamel junction (CEJ) using a diamond rotary disc, and the dental pulp was removed. The pulp tissue was rinsed in α -minimum essential medium (α -MEM) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich) and antibiotics (100 units/mL penicillin G and 100 μ g/mL streptomycin; Invitrogen Life Technologies; Carlsbad, California, United States) and minced into fragments of 1 to 2 mm³ before being placed in a 37°C humidified tissue culture incubator at 5% CO₂ for 4 days. The culture medium was changed every 3 days. When reaching 80% confluence, cells were harvested by using 0.05% trypsin-EDTA solution (Sigma-Aldrich) and subculture being performed for the experiments. DPSCs between passage 2 and 4 were used for the experiments.¹³ To prove that the cells obtained were MSC, CD105, and CD45 expression were examined.¹⁴

Examination of DPSCs Proliferation Ability

4 × 10⁴ DPSCs was cultured in 96-well tissue culture using α -MEM medium, 10% FBS (Sigma-Aldrich) and antibiotics (100 units/mL penicillin G and 100 μ g/mL streptomycin). Five μ g/ml and 10 μ g/mL of ConA were added in the treatment group for 24 hours. However, ConA was not added to the control group culture. After 48 hours of culture, a 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) test was performed. 0.5 mg/mL of MTT was added to each well and incubated for 4 hours at 37°C and 5% CO₂. Dimethyl sulfoxide was added to each well and optical density (OD) was examined using an enzyme-linked immunosorbent assay reader (Bio-Rad; California, United States) at a wavelength of 550 nm.¹⁵

Examination of DPSCs Osteogenic Differentiation Ability

A total of 1 × 10⁵ DPSCs was cultured in 24-well tissue culture using Dulbecco's Modified Eagle Medium (DMEM) medium plus 10% FBS and antibiotics. After confluence, the medium was replaced with DMEM plus 10% FBS, 10mM-glycerophosphate, 100 nM dexamethasone, and 50 μ g/mL ascorbic acid-2-phosphate (osteogenic medium). Five μ g/ml and 10 μ g/mL of ConA were added to the treatment group, but not to the control group. The cells were incubated at 37°C in 5% CO₂. After 28 days, the culture medium was aspirated and the cell layer washed twice with PBS, and fixed with 95% ethanol for 10 minutes at room temperature. They were then washed twice with distilled water and stained for 30 minutes with 1% alizarin red S solution (Sigma-Aldrich; Merck KGaA) at room temperature. Photographs were taken after the cell layer had been washed with water using Canon Power Shot G12 digital camera (Tokyo, Japan).¹⁵

Data Analysis

Data were analyzed by means of a two-way analysis of variance (ANOVA) and expressed as mean \pm standard deviation. The statistical differences between the two groups were evaluated using a Student's *t*-test. In all the analyses, *p* < 0.05 was considered to indicate a statistically significant difference.

Results

DPSCs Isolation from the Pulp of Third Molars

DPSCs appeared to be morphologically fibroblastic and after changing the culture media every 3 days, the number of DPSCs increased to the extent that on day 7 they appeared confluent (**Fig. 1A**). To ensure that the cells were stem cells, CD 105 and CD 45 expression was examined. DPSC expresses CD 105 but not CD 45 (**Fig. 1B**).

DPSCs Proliferation Ability after Addition of ConA

The effect of adding ConA to the proliferation of DPSCs was analyzed by MTT assay. The addition of 5 and 10 μ g/mL of ConA can significantly increase the proliferation of DPSCs (*p* \leq 0.05). A concentration of 10 μ g/mL produced higher proliferation ability than 5 μ g/mL (**Fig. 2**).

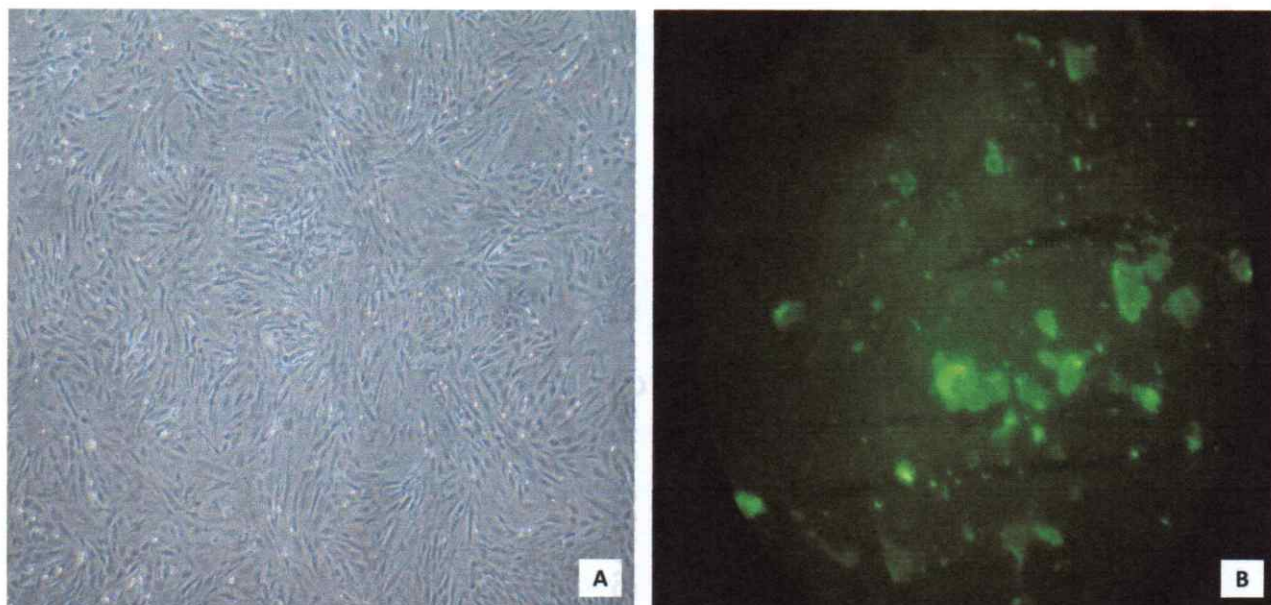


Fig. 1 Dental pulp stem cells culture on the 7th day, a fibroblastic-like cell appears attached to the base of the petri dish (A) and expresses CD105 (B).

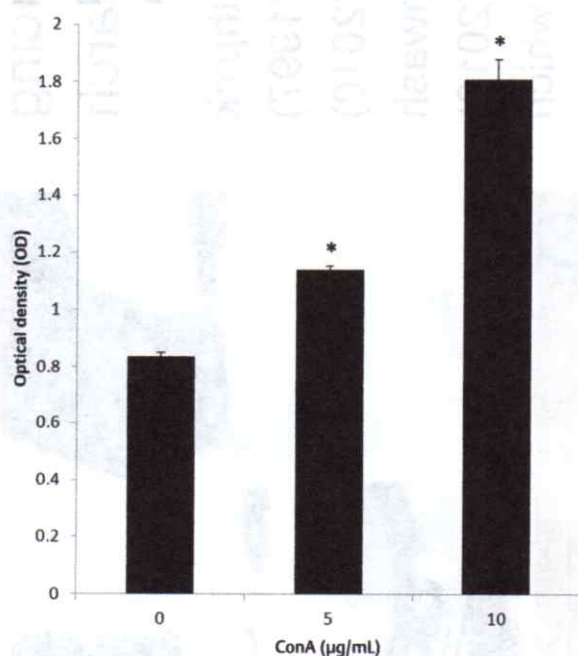


Fig. 2 ConA can increase the proliferation of DPSCs. DPSCs were cultured in osteogenic medium with 5 and 10 µg/mL ConA being added for 24 hours. An MTT assay was performed to measure cell proliferation. ConA at concentrations of 5 and 10 µg/mL significantly increased the proliferation of DPSCs. The data are mean ± standard deviation for four cultures. * $p < 0.05$ as compared with the control. ConA, concanavalin A; DPSC, dental pulp stem cell; MTT, 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide.

DPSCs Osteogenic Differentiation Ability after the Addition of ConA

To evaluate the effect of ConA on osteogenic differentiation of DPSCs, culture staining was performed with alizarin red on day

21. It was observed that the addition of 5 and 10 µg/mL of CoA increased the osteogenic differentiation of DPSCs characterized by mineralization. A concentration of 10 µg/mL produced higher osteogenic differentiation ability than 5 µg/mL (→ Fig. 3).

Discussion

Stem cells are a new alternative for regenerating pulp tissue. Dental pulp tissue is a very promising source of MSC in regenerative dentistry because it has multidifferentiation properties, isolation processes that are noninvasive and efficient, immunosuppressive properties and similarities with osteoblasts.¹⁶⁻¹⁸ DPSCs can differentiate into odontoblasts in vivo. DPSCs are capable of regenerating dental-pulp-like complex by forming reparative dentin-like structures on the surface of dentin.^{2,5,19} For regeneration therapy using stem cells, a considerable number of cells are required. Because the number of stem cells in the body is very limited, a method to increase their number without eliminating their differentiation capabilities must be developed. In this study, ConA was used to improve the proliferation and osteogenic differentiation of DPSCs.

In this study, ConA can increase the proliferation of DPSCs in vitro. Lectins have been shown to exert a strong effect on the proliferation and differentiation of various animal cells, including lymphocytes, osteoblasts, and chondrocytes.^{10,11,20,21} ConA induces changes in the morphology and proliferation of lymphocyte cells. In chondrocyte cell culture, ConA induces changes from fibroblastic cells to spherical cells, while also increasing aggrecan synthesis within 24 hours. This effect is greater than those of growth factors and hormones.^{10,20} In the other hand, increased proliferation of DPSCs due to the addition of ConA does not occur in tumor cells. Lectins can inhibit proliferation and have a cytotoxic effect on tumor cells.²² Moreover, ConA induces apoptosis in human melanoma

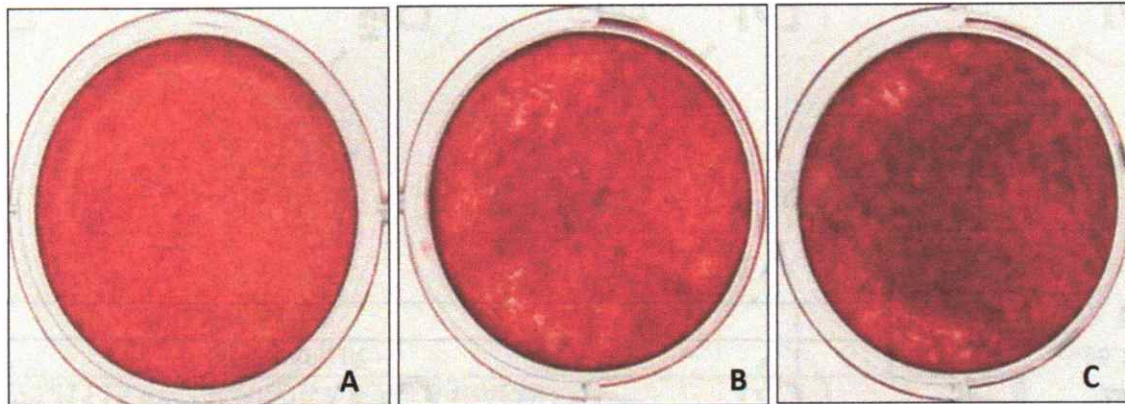


Fig. 3 ConA can increase osteogenic differentiation of DPSCs. DPSCs cultures are maintained in the osteogenic medium (A) or in osteogenic medium with 5 (B) and 10 µg/mL of ConA (C). The cell layers were stained with alizarin red on day 21. ConA, concanavalin A; DPSC, dental pulp stem cell.

cells. Subsequently, ConA caused mitochondrial transmembrane potential collapse, cytochrome c release, activation of caspases, and eventually triggering a mitochondria-mediated apoptosis.²³

In this study, it was also found that ConA can increase mineralization in DPSCs cultures which are placed in an osteogenic medium. This finding is consistent with those of previous studies that the addition of ConA can improve the process of MSC mineralization or calcification.¹² The increase in osteogenic differentiation of DPSCs is probably due to ConA increasing BMP-2 levels in DPSCs culture. BMP-2 is an inductive growth factor for osteogenic differentiation of various stem cells.²⁴ ConA can increase levels of osteocalcin and BMP-2 proteins in MSC culture. In addition, ConA increases ALP, Runx2, osteocalcin, BMP2, BMP4, and BMP-6 at the level of mRNA expression.¹²

Conclusion

From this study, it can be concluded that ConA can increase proliferation, maintain, and enhance osteogenic differentiation of DPSCs in vitro. Further studies should be performed to understand the signaling pathway underlying the effects of ConA on proliferation and osteogenic differentiation of DPSCs.

Conflict of Interest

None declared.

References

- 1 Ponnaiyan D, Jegadeesan V. Comparison of phenotype and differentiation marker gene expression profiles in human dental pulp and bone marrow mesenchymal stem cells. *Eur J Dent* 2014;8(3):307–313
- 2 Gronthos S, Mankani M, Brahmi J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci U S A* 2000;97(25):13625–13630
- 3 Arthur A, Rychkov G, Shi S, Koblar SA, Gronthos S. Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues. *Stem Cells* 2008;26(7):1787–1795

- 4 Sugiyama M, Iohara K, Wakita H, et al. Dental pulp-derived CD31/CD146⁺ side population stem/progenitor cells enhance recovery of focal cerebral ischemia in rats. *Tissue Eng Part A* 2011;17(9-10):1303–1311
- 5 Miura M, Gronthos S, Zhao M, et al. SHED: stem cells from human exfoliated deciduous teeth. *Proc Natl Acad Sci USA* 2003;100(10):5807–5812
- 6 Laino G, Graziano A, d'Aquino R, et al. An approachable human adult stem cell source for hard-tissue engineering. *J Cell Physiol* 2006;206(3):693–701
- 7 d'Aquino R, Graziano A, Sampaolesi M, et al. Human postnatal dental pulp cells co-differentiate into osteoblasts and endothelial cells: a pivotal synergy leading to adult bone tissue formation. *Cell Death Differ* 2007;14(6):1162–1171
- 8 Simpson AH, Mills L, Noble B. The role of growth factors and related agents in accelerating fracture healing. *J Bone Joint Surg Br* 2006;88(6):701–705
- 9 Hanada K, Dennis JE, Caplan AI. Stimulatory effects of basic fibroblast growth factor and bone morphogenetic protein-2 on osteogenic differentiation of rat bone marrow-derived mesenchymal stem cells. *J Bone Miner Res* 1997;12(10):1606–1614
- 10 Yan W, Pan H, Ishida H, et al. Effects of concanavalin A on chondrocyte hypertrophy and matrix calcification. *J Biol Chem* 1997;272(12):7833–7840
- 11 Oda R, Suardita K, Fujimoto K, et al. Anti-membrane-bound transferrin-like protein antibodies induce cell-shape change and chondrocyte differentiation in the presence or absence of concanavalin A. *J Cell Sci* 2003;116(Pt 10):2029–2038
- 12 Sekiya K, Nishimura M, Suehiro F, Nishimura H, Hamada T, Kato Y. Enhancement of osteogenesis by concanavalin A in human bone marrow mesenchymal stem cell cultures. *Int J Artif Organs* 2008;31(8):708–715
- 13 Hilken P, Gervois P, Fanton Y, et al. Effect of isolation methodology on stem cell properties and multilineage differentiation potential of human dental pulp stem cells. *Cell Tissue Res* 2013;353(1):65–78
- 14 Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytherapy* 2006;8(4):315–317
- 15 Byun YK, Kim KH, Kim SH, et al. Effects of immunosuppressants, FK506 and cyclosporin A, on the osteogenic differentiation of rat mesenchymal stem cells. *J Periodontol* 2012;42(3):73–80
- 16 Bakopoulou A, Apatzidou D, Aggelidou E, et al. Isolation and prolonged expansion of oral mesenchymal stem cells under

- clinical-grade, GMP-compliant conditions differentially affects "stemness" properties. *Stem Cell Res Ther* 2017;8(1):247
- 17 Yang H, Li J, Sun J, et al. Cells isolated from cryopreserved dental follicle display similar characteristics to cryopreserved dental follicle cells. *Cryobiology* 2017;78:47–55
 - 18 Rodas-Junco BA, Villicaña C. Dental pulp stem cells: current advances in isolation, expansion and preservation. *Tissue Eng Regen Med* 2017;14(4):333–347
 - 19 Batouli S, Miura M, Brahim J, et al. Comparison of stem-cell-mediated osteogenesis and dentinogenesis. *J Dent Res* 2003;82(12):976–981
 - 20 Yan WQ, Nakashima K, Iwamoto M, Kato Y. Stimulation by concanavalin A of cartilage-matrix proteoglycan synthesis in chondrocyte cultures. *J Biol Chem* 1990;265(17):10125–10131
 - 21 Nakamasu K, Kawamoto T, Shen M, et al. Membrane-bound transferrin-like protein (MTF): structure, evolution and selective expression during chondrogenic differentiation of mouse embryonic cells. *Biochim Biophys Acta* 1999;1447(2-3):258–264
 - 22 De Mejía EG, Prisecaru VI. Lectins as bioactive plant proteins: a potential in cancer treatment. *Crit Rev Food Sci Nutr* 2005;45(6):425–445
 - 23 Liu B, Li CY, Bian HJ, Min MW, Chen LF, Bao JK. Antiproliferative activity and apoptosis-inducing mechanism of Concanavalin A on human melanoma A375 cells. *Arch Biochem Biophys* 2009;482(1-2):1–6
 - 24 Chen D, Zhao M, Mundy GR. Bone morphogenetic proteins. *Growth Factors* 2004;22(4):233–241



Thieme

Author Queries

- Q1:** Please confirm that given names (red), middle names (black) and surnames (green) have been identified correctly. Author names in bibliographic citations and online will appear as: Suardita K, Arundina I, Tedjosongko U, Yuliati A, Peeters HH, Wijaksana IKE, Surboyo MDC. Concanavalin A Enhanced Proliferation and Osteogenic Differentiation of Dental Pulp Stem Cells. Please confirm if this is correct.
- Q2:** Please provide division/department for affiliation 5, if any.
- Q3:** Please provide the academic degree(s) of corresponding author.



Thieme