

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

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## 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.<sup>1</sup> DPSCs are multipotent and can differentiate into chondrocytes, adipocytes, osteoblasts/osteocytes, myocytes, nerve cells, cardiomyocytes, and odontoblasts.<sup>2-4</sup> 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.<sup>2,5-7</sup> With the ability to differentiate into odontoblast, DPSCs can

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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- $\beta$ 1 (TGF- $\beta$ 1).<sup>8</sup> 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.<sup>9</sup> 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.<sup>10,11</sup> 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.<sup>12</sup> 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  $\alpha$ -minimum essential medium ( $\alpha$ -MEM) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich) and antibiotics (100 units/mL penicillin G and 100  $\mu$ g/mL streptomycin; Invitrogen Life Technologies; Carlsbad, California, United States) and minced into fragments of 1 to 2 mm<sup>3</sup> before being placed in a 37°C humidified tissue culture incubator at 5% CO<sub>2</sub> 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.<sup>13</sup> To prove that the cells obtained were MSC, CD105, and CD45 expression were examined.<sup>14</sup>

### Examination of DPSCs Proliferation Ability

4 × 10<sup>4</sup> DPSCs was cultured in 96-well tissue culture using  $\alpha$ -MEM medium, 10% FBS (Sigma-Aldrich) and antibiotics (100 units/mL penicillin G and 100  $\mu$ g/mL streptomycin). Five  $\mu$ g/ml and 10  $\mu$ 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% CO<sub>2</sub>. 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.<sup>15</sup>

### Examination of DPSCs Osteogenic Differentiation Ability

A total of 1 × 10<sup>5</sup> 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  $\mu$ g/mL ascorbic acid-2-phosphate (osteogenic medium). Five  $\mu$ g/ml and 10  $\mu$ 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% CO<sub>2</sub>. 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).<sup>15</sup>

### 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.

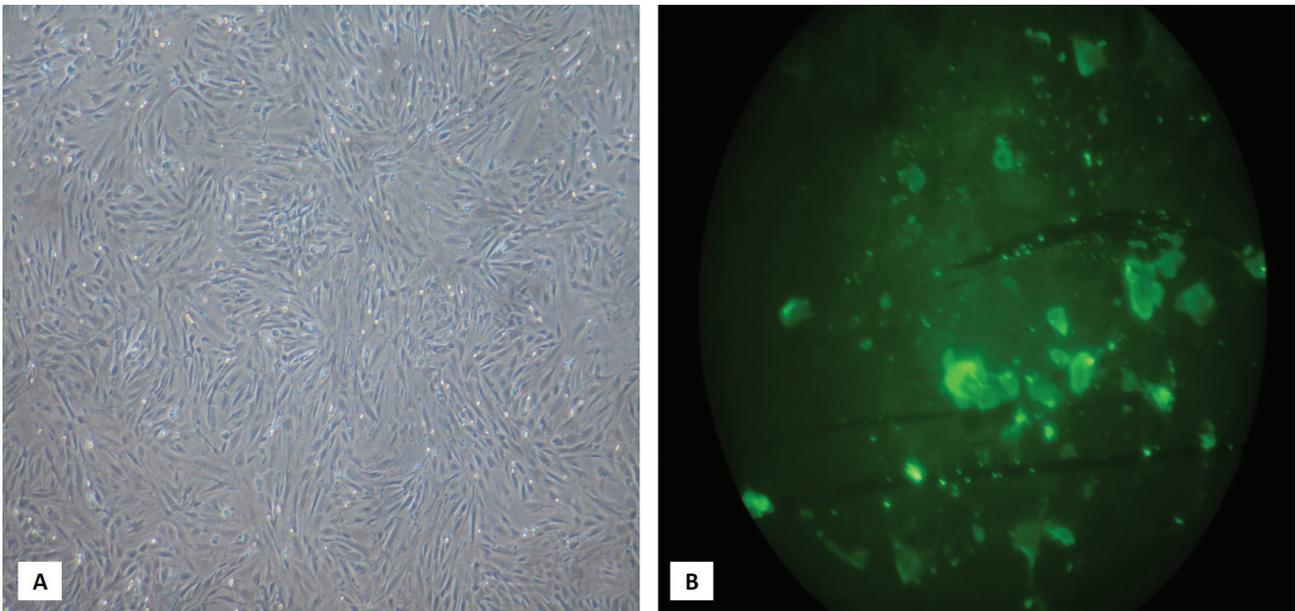
## Results

### DPSCs Isolation from the Pulp of Third Molars

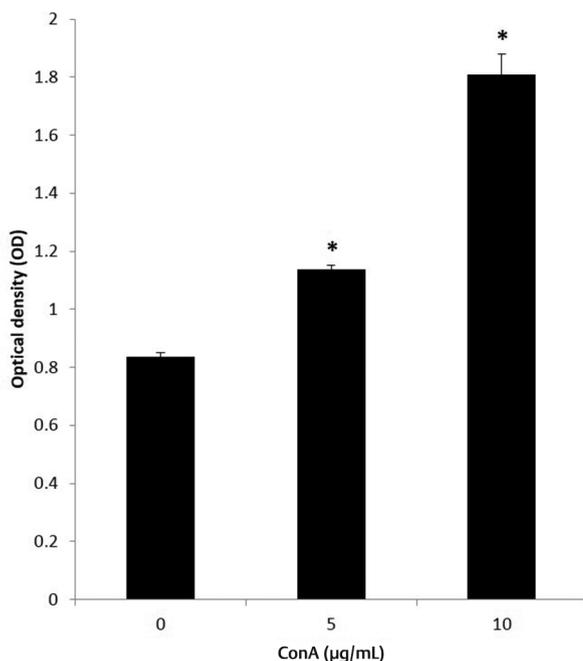
DPSCs appeared to be morphologically fibroblastic and after changing the culture media every 3 days, the number of DPSCs increased to the extent that on day 7 they appeared confluent (**Fig. 1A**). To ensure that the cells were stem cells, CD 105 and CD 45 expression was examined. DPSC expresses CD 105 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  $\mu$ g/mL of ConA can significantly increase the proliferation of DPSCs (*p*  $\leq$  0.05). A concentration of 10  $\mu$ g/mL produced higher proliferation ability than 5  $\mu$ 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

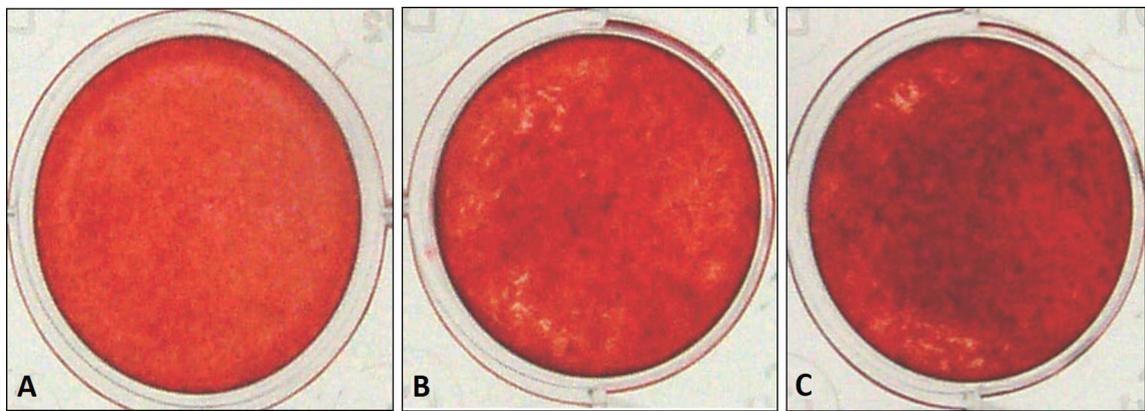
To evaluate the effect of ConA on osteogenic differentiation of DPSCs, culture staining was performed with alizarin red on day

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.<sup>16-18</sup> 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.<sup>2,5,19</sup> 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.<sup>10,11,20,21</sup> 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.<sup>10,20</sup> 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.<sup>22</sup> 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.<sup>23</sup>

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.<sup>12</sup> 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.<sup>24</sup> 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.<sup>12</sup>

## 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.

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