

# Isolation and characterization of dental pulp stem cells from a supernumerary tooth

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**BACKGROUND:** Dental pulp stem cells (DPSCs) were primarily derived from the pulp tissues of primary incisors and permanent third molar teeth, whereas no report to our knowledge has yet been documented on deriving DPSCs from the other tooth types. The aim of this study is to present a novel approach of harvesting stem cells from a supernumerary tooth (a mesiodens).

**MATERIALS AND METHODS:** The pulp tissues from a mesiodens of a 20-year-old healthy male patient and the left lower deciduous canine of a healthy 10-year-old boy (the positive control) were extracted and cultured for DPSCs, which were examined with stem cells (Oct-4, Nanog and Rex-1) and differentiation (Osteonectin and Nestin) markers. Furthermore, DPSCs were directionally differentiated to osteogenic and adipogenic cell lineages.

**RESULTS:** Dental pulp stem cells derived from the mesiodens were capable of differentiating into adipogenic and osteogenic lineages. The mesiodens's DPSCs also expressed stem cell and differentiation markers, which suggested their stem cell origin and differentiation capability. All the aforementioned results for the mesiodens were consistent with those of the DPSCs derived from the positive control.

**CONCLUSION:** We have demonstrated the feasibility of deriving DPSCs from a usually discarded tissue such as a supernumerary tooth.

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**Keywords:** dental pulp stem cell; supernumerary tooth

## Introduction

Dental pulp stem cells (DPSCs) share similar gene expression profiles and differentiation capability to that of bone marrow derived stem cells (BMSCs) (1, 2). DPSCs are potentially superior to other types of adult stem cell as teeth are easy to access and are extracted

routinely throughout life. Even of more importance is the ability to secure DPSCs at a young age and store it for the future usage. A personalized stem cell can then be created from DPSC without using procedures that may cause ethical concerns.

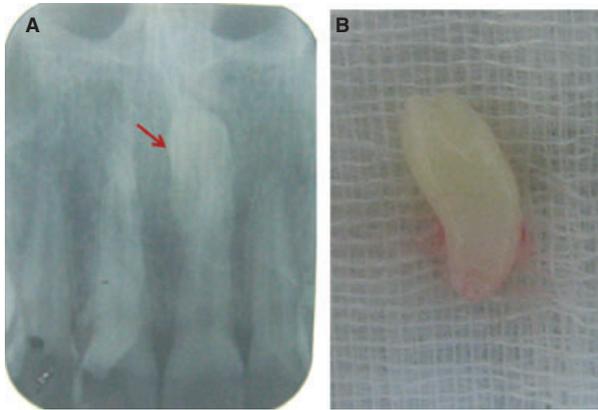
Previous researches on DPSCs were primarily focused on the application of pulp tissues from healthy primary incisors and permanent third molar teeth (3). There was no report to our knowledge about the derivation of DPSCs from the other tooth types. The incidence of supernumerary teeth varies between 0.45% and 3% depending on the literature source (4). Here, we presented our recent success in the isolation and characterization of DPSCs from the pulp tissue of a supernumerary tooth.

## Materials and methods

A healthy 20-year-old-male patient required the extraction of a supernumerary tooth (a mesiodens) (Fig. 1A,B). A deciduous tooth (left lower canine) was also removed from a healthy 10-year-old boy because of high mobility for positive control. Both the mesiodens and deciduous tooth were removed using local anesthesia with the consent of the patient (for mesiodens) and the patient's parents (for deciduous tooth). Both teeth (mesiodens and deciduous tooth) were kept on ice in Dulbecco's phosphate buffered saline (DPBS; Invitrogen, Carlsbad, CA, USA) and delivered to the laboratory for the isolation of DPSCs. The surfaces of both teeth were first cleaned with DPBS and a groove of 0.5–1.0 mm deep was cut around the circumference of the teeth using a sterile hand-held high-speed drill. The dental pulps were exposed by splitting the teeth with a chisel along the groove. The pulp tissues of both teeth were then extracted with endodontic files.

The extracted pulp tissues were subsequently digested in collagenase type I (3 mg/ml, Invitrogen) and dispase (4 mg/ml, Invitrogen) for 1 h at 37°C (1). The resulted cell suspensions from both teeth were filtered through a 40- $\mu$ m cell strainer (Falcon, BD, Franklin Lakes, NJ, USA), and the single cell suspensions were then cultured in a DPSC medium containing  $\alpha$ -modified Eagle's

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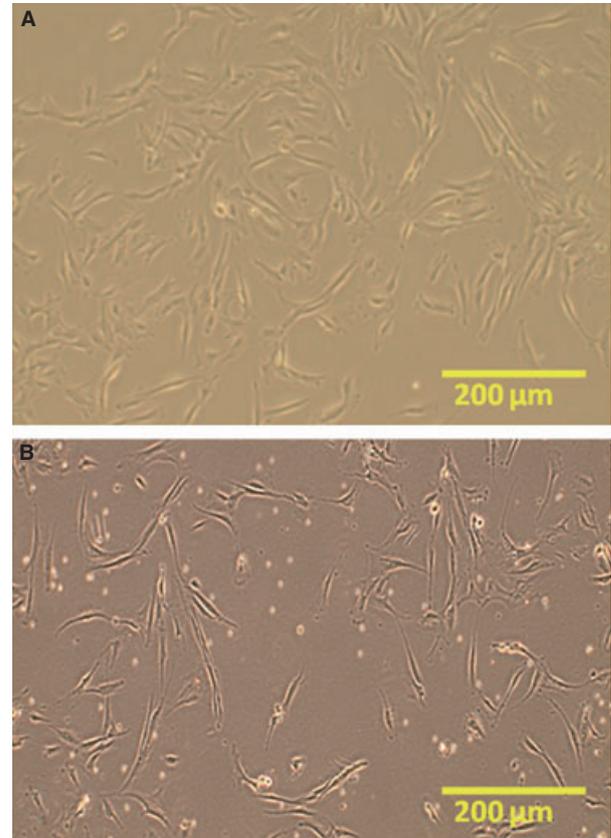


**Figure 1** (A) The periapical radiography of the upper anterior region of the patient in this study revealing a mesiodens, as indicated by the arrow. (B) The removed mesiodens.

medium (Hyclone, Logan, UT, USA), 20% fetal bovine serum (Hyclone), 100  $\mu$ M L-ascorbic acid-2-phosphate (Sigma, St Louis, MO, USA), 100 units/ml penicillin (Sigma), and 100  $\mu$ g/ml streptomycin (Invitrogen). DPSCs were cultured at 37°C with 5% CO<sub>2</sub>. Fresh medium was replaced every 2 days and at 100% confluence for optimal cell harvesting.

Third passage DPSCs from both the mesiodens and deciduous tooth were seeded in a 6-well plate at 40 single cells/well with three replicas. After 2 weeks in culture, the cells were fixed in 10% buffered formalin for 10 min and stained with 3% (v/v) crystal violet (Sigma) for 5 min. The cells were washed twice with distilled water and the number of colonies was counted. Colonies greater than 2 mm in diameter were enumerated, if it was too small it could be the fall-off cell or satellite cell that was growing instead of the cell originally seeded. The percentage colony forming efficiency was expressed as the total number of colonies divided by the initial number of cells that were seeded and multiplied by 100 (5). As a result, 72% of the DPSCs derived from mesiodens and 83% of the DPSCs from deciduous tooth were capable of forming colonies.

To differentiate adipogenic lineage, the third passage DPSCs were seeded at 400 cells/cm<sup>2</sup> in a 35-mm tissue culture dish and cultured for 11 days in a DPSC medium until confluence was achieved. The DPSC medium was subsequently supplemented with 5  $\mu$ g/ml



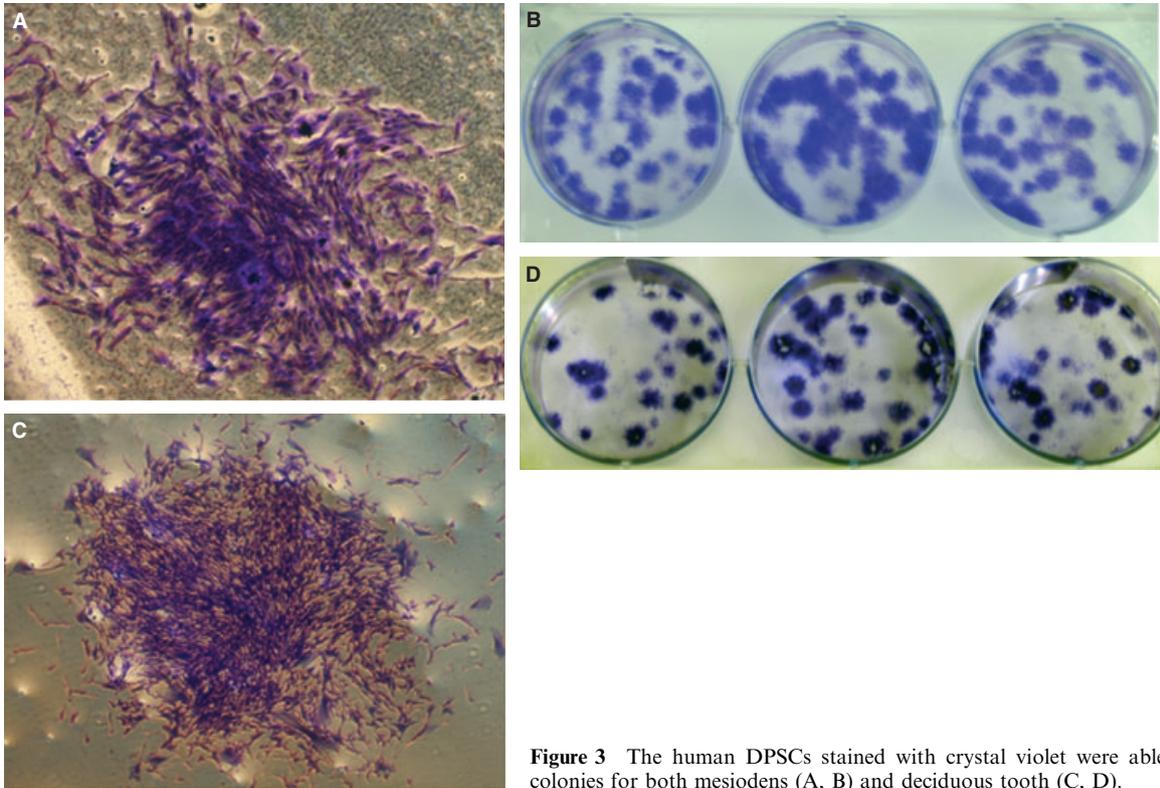
**Figure 2** The human DPSCs from both the mesiodens (A) and deciduous tooth (B) were spindle-shaped in morphology.

insulin (Sigma), 50  $\mu$ M indomethacin (Sigma), 1  $\mu$ M dexamethasone (Sigma), and 0.5  $\mu$ M isobutyl-1-methyl xanthine (Sigma). The cultures were then placed in an incubator at 37°C and 5% CO<sub>2</sub> for another 21 days with medium changes, three times per week. As control, DPSC medium only was used. At the end of the cultivation period, the cells were then fixed in 10% buffered formalin for 10 min and stained with oil-red-O (Sigma) to stain lipid vacuoles (5). To differentiate osteogenic lineage, the cells were prepared as described except that 1 nM dexamethasone (Sigma), 20 mM  $\beta$ -glycerolphosphate (Sigma), and 50 ng/ml L-thyroxine sodium pentahydrate (Sigma) were supplemented in the DPSC medium on day 11. The culture was incubated at

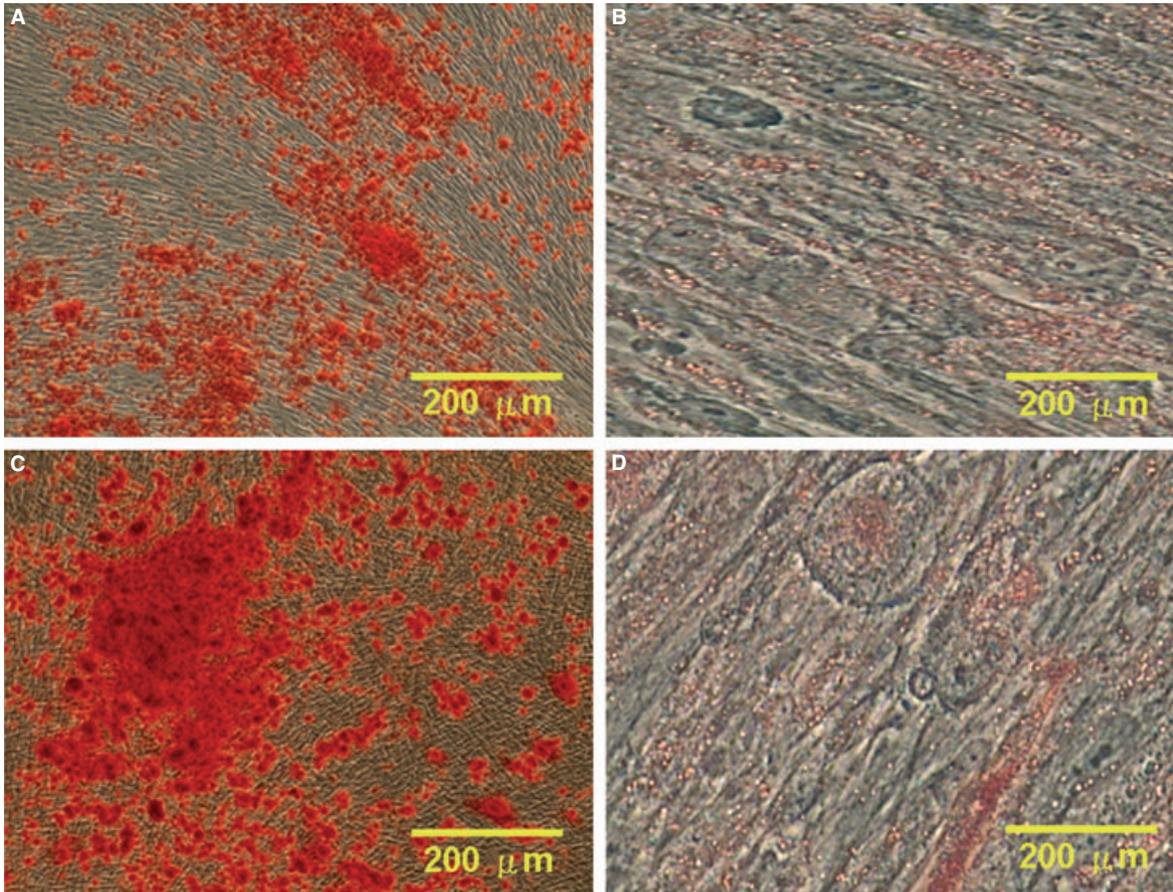
**Table 1** Specific primer sets targeting for stem cell and differentiation markers as well as GAPDH used as control

Primers	Sequences	PCR products
Oct4	Forward: 5'-AAG AAC ATG TGT AAG CTG CGG CCC-3' Reverse: 5'-GGA AAG GCT TCC CCC TCA GGG AAA GG-3'	455-bp
Rex-1	Forward: 5'-AGA ATT CGC TTG AGT ATT CTG A-3' Reverse: 5'-GGC TTT CAG GTT ATT TGA CTG A-3'	470-bp
Nanog	Forward: 5'-TCT CTC CTC TTC CTT CCT CCA-3' Reverse: 5'-GGA AGA GTA GAG GCT GGG GT-3'	389-bp
Nestin	Forward: 5'-TGG CAA GGC GAC TGG GCG A-3' Reverse: 5'-CCC TCT ATG GCT GTT TCT TTC TCT-3'	810-bp
Osteonectin	Forward: 5'-ATC TTC TTT CTC CTT TGC CTG G-3' Reverse: 5'-GCA CAC CTC TCA AAC TCG CC-3'	323-bp
GAPDH	Forward: 5'-ATG GGG AAG GTG AAG GTC GG-3' Reverse: 5'-CCA TCA CGC CAC AGT TTC CC-3'	596-bp

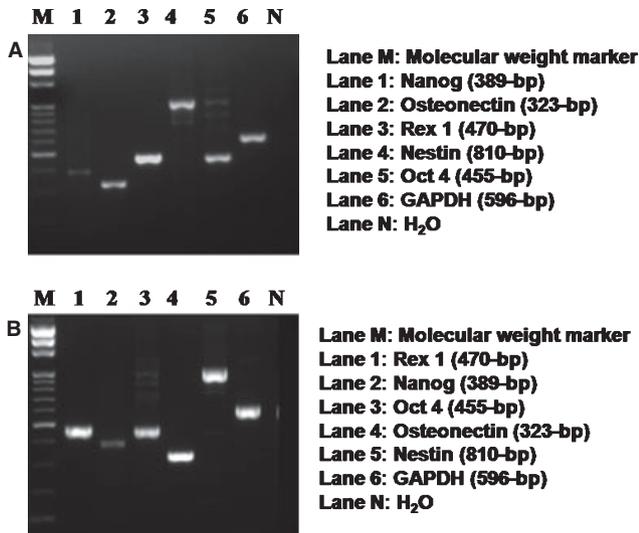
GAPDH, glyceraldehyde-3-phosphate dehydrogenase.



**Figure 3** The human DPSCs stained with crystal violet were able to form colonies for both mesiodens (A, B) and deciduous tooth (C, D).



**Figure 4** Dental pulp stem cells of mesiodens were able to differentiate between adipogenic (A) and osteogenic lineage (B), whereas DPSCs of the deciduous tooth were also able to differentiate between osteogenic (C) and adipogenic lineage (D).



**Figure 5** Dental pulp stem cells of mesiodens (A) and deciduous tooth (B) expressed the stem cell (Oct-4, Nanog and Rex-1) and differentiation (Osteonectin and Nestin) markers upon reverse transcription polymerase chain reaction. Glyceraldehyde-3-phosphate dehydrogenase was the positive control whereas H<sub>2</sub>O was the negative control.

37°C and 5% CO<sub>2</sub> for another 21 days with medium changes, three times per week. As control, DPSC medium only was used. At the end of the cultivation period, DPSCs of both mesiodens and deciduous tooth were fixed in 10% buffered formalin for 10 min and stained with alizarin red S (Wako, Osaka, Japan) for 15 min at room temperature that the mineral matrix of bone could be observed (5).

In addition, we have determined if DPSCs of both mesiodens and deciduous tooth shared similar expression pattern of stem cell and differentiation markers as previously described in DPSCs (1) and BMSCs (2) by the reverse transcription-polymerase chain reaction carried out on a DNA thermal cycler (Takara MP, Tokyo, Japan). First, total RNA was obtained by the RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) followed by reverse transcription of the mRNA according to the procedure provided by the SuperScript III (Invitrogen). The resulting cDNA was then used for PCR amplification. The PCR reactions were performed using specific primer sets (6) which were designed on the basis of the published cDNA sequences from National Center for Biotechnology Information. The amplification cycle was 3 min at 94°C, 25 s at 94°C, 30 s at 62°C, and 50 s at 72°C, which was repeated 40 times followed by 3 min at 72°C. Five primer sets were designed to determine the expression of stem cell markers (Oct4, Rex-1 and Nanog; Table 1) and differentiation markers (Nestin and Osteonectin; Table 1), whereas glyceraldehyde-3-phosphate dehydrogenase was used as the positive control (Table 1) and H<sub>2</sub>O was the negative control.

## Results

Dental pulp stem cells from both the mesiodens and deciduous tooth proliferated rapidly with spindle shape

morphology (Fig. 2A,B). Following the third passage, a homogenous population of DPSCs of both teeth was acquired and maintained in the culture based on cell morphology. We found that 72% of the DPSCs derived from mesiodens (Fig. 3A,B) and 83% of the DPSCs from deciduous tooth (Fig. 3C,D) were capable of forming colonies. Furthermore, DPSCs of both mesiodens and deciduous tooth were successful in differentiating into adipogenic (Fig. 4A,B) and osteogenic (Fig. 4C,D) lineages. Also, we have determined that DPSCs of both mesiodens and deciduous tooth expressed stem cell and differentiation markers (Fig. 5A,B).

## Discussion

The derivation of DPSCs was first described in 2000 (1). They share similar expression profiles and differentiation capabilities to that of BMSCs (1, 2). Most of the prior studies on DPSCs are focused on primary incisors and permanent third molar teeth (1); here, we demonstrated the feasibility of deriving DPSCs from a supernumerary tooth (a mesiodens). Being able to isolate DPSCs from a supernumerary tooth, as demonstrated in this report, we could extend the potential and possibility of the source of DPSCs. Furthermore, DPSCs would be the most convenient source of stem cells because teeth were easy to retrieve and removed throughout life.

In conclusion, this report has described the successful isolation and characterization of DPSCs from the pulp tissue of a mesiodens. Supernumerary teeth usually discarded after extraction might therefore represent a valuable source of human DPSCs.

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