Neurogenic differentiation of dental pulp stem cells to neuron-like cells in dopaminergic and motor neuronal inductive media

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KEYWORDS
dental pulp stem cells; dopaminergic neuronal inductive media; motor neuronal inductive media; neuron-like cells

Background/purpose: Dental pulp stem cells (DPSCs) have been proposed as a promising source of stem cells in nerve regeneration due to their close embryonic origin and ease of harvest. The aim of this study was to evaluate the efficacy of dopaminergic and motor neuronal inductive media on transdifferentiation of human DPSCs (hDPSCs) into neuron-like cells.

Methods: Isolation, cultivation, and identification of hDPSCs were performed with morphological analyses and flow cytometry. The proliferation potential of DPSCs was evaluated with an XTT [(2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) assay. Media for the induction of dopaminergic and spinal motor neuronal differentiation were prepared. The efficacy of neural induction was evaluated by detecting the expression of neuron cell-specific cell markers in DPSCs by immunocytochemistry and quantitative real-time reverse transcription polymerase chain reaction (RT-PCR).

Results: In the XTT assay, there was a 2.6- or 2-fold decrease in DPSCs cultured in dopaminergic or motor neuronal inductive media, respectively. The proportions of βIII-tubulin (βIII-tub), glial fibrillary acidic protein (GFAP), and oligodendrocyte (O1)-positive cells were significantly higher in DPSCs cultured in both neuronal inductive media compared with those cultured in control media. Furthermore, hDPSC-derived dopaminergic and spinal motor neuron cells after induction expressed a higher density of neuron cell markers than those prior to induction.

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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Introduction

Nerve degeneration or damage may cause severe complications leading to severe disability in patients. The use of stem cell therapy for nerve regeneration may be an important strategy for treatment. Mesenchymal stem cells (MSCs) or bone marrow stem cells (BMSCs) may be used as the source of stem cells for nerve regeneration treatment. Several studies have demonstrated the ability of MSCs to transdifferentiate into functional cells of the nervous system. MSCs cultured in neural stem cell (NSC) culture conditions can further differentiate into astrocytes, neurons, and oligodendrocytes. However, the transdifferentiating efficacy of MSCs and BMSCs is low and unpredictable.

Recently, dental pulp cells (DPCs) and dental pulp stem cells (DPSCs) have been proposed as a promising source of stem cells in nerve regeneration due to their close embryonic origin and ease of harvest. DPSCs are a source of progenitor/stem cells that can proliferate and differentiate into multiple cell lineages in vitro. Interestingly, DPSCs originate from the cranial neural crest and have neural characteristics. The dental pulp tissue is termed "ectomesenchyme", because it derives from ectodermal cells growing on the periphery of the neural tube during embryonic development, migrating into the oral region, and transdifferentiating into a mesenchymal phenotype. MSCs are immune-privileged and their propagation is not challenged by ethical concerns. Therefore, DPSCs may represent a promising source in cell therapy for neurological disorders.

DPCs can significantly promote survival of embryonic tyrosine hydroxylase (TH)-positive neurons in culture, because DPCs can release some neurotrophic factors. In animal studies, grafting of dental pulp tissue into hemicontinuous spinal cord increases the number of surviving motor neurons. Neurotrophic factors secreted from the implanted dental pulp tissue are considered to be the main contributor towards the rescuing effect on motor neurons. Even under non-neuronal inductive conditions, human adult DPCs and DPSCs can express neural progenitor marker nestin and glial marker glial fibrillary acidic protein (GFAP) at both the gene and protein levels. DPSCs can differentiate into a variety of cell lineages, including adipocytes, odontoblasts, neural cells, and glial cells. Following ex vivo expansion, such multipotential DPSCs still retain their neural crest properties. Under neural inductive conditions, DPSCs can express the postmitotic neuron-specific marker neuronal nuclei (NeuN), which indicates their potential in neural differentiation.

Although DPSCs in media supplemented with differentiation factors can differentiate into neuron-like cells, their differentiations are incomplete. Previous studies compared murine DPCs with murine retinal progenitor cells to evaluate their potential for differentiation into neuron-like cells. The differentiation of the murine DPCs into glial- and neuron-like cells was incomplete. After differentiation, 37% of murine retinal progenitor cells, but only 5% of murine DPCs, expressed the astroglia cell-marker GFAP. We hypothesized that DPSCs exposed to the appropriate environmental conditions would differentiate into functionally active neurons. Moreover, neural crest-derived adult DPSCs might provide an alternative stem cell source for therapy-based treatments for neurological disorders and injury.

This study examined the neuronal differentiation potential of adult human DPSCs (hDPSCs) in vitro. The aim of this study was to evaluate the neuronal differentiation potential of hDPSCs into neuron-like cells in both dopaminergic and motor neuronal inductive media containing growth factors such as brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), basic fibroblast growth factor (bFGF), and retinoic acid. The expression of neuron cell-specific cell markers in hDPSCs cultured in neuronal induction media was assessed by immunocytochemistry and quantitative real-time reverse transcription polymerase chain reaction (RT-PCR).

Methods

Materials

Dulbecco’s modified Eagle’s medium (DMEM) and F12 media (DMEM/F12), fetal calf serum (FCS), N2 supplements, penicillin, and streptomycin were obtained from Invitrogen (Carlsbad, CA, USA); insulin-like growth factor (IGF)-1 and transforming growth factor (TGF)-IIIb from Promega and TGF-IIIb from Promega; Sonic hedgehog, FGF-8b were purchased from Promega; and other cytokines were purchased from PeproTech Systems (Rocky Hill, NJ, USA). Goat polyclonal anti-choline acetyltransferase antibody (anti-ChAT), rabbit polyclonal anti-GFAP, rabbit polyclonal anti-dopa decarboxylase (anti-DDC), mouse monoclonal anti-tyrosine hydroxylase (anti-TH), mouse monoclonal anti-neuronal nuclei (anti-NeuN), mouse monoclonal anti-ß-III-tubulin (anti-ß-III-tubl), and mouse monoclonal anti-oligodendrocyte (anti-O1) were purchased from Chemicon-Millipore (Billerica, MA, USA); poly-L-lysine (PLL) was obtained from ScienCell Research Laboratories (Carlsbad, CA, USA). GAPDH was purchased from Mission Biotech Co. (Taipei, Taiwan), collagenase type I, dispase, L-glutamine, non-essential amino acids were purchased from Invitrogen (Carlsbad, CA, USA). Sonic hedgehog, FGF-8b were purchased from PeproTech Systems (Rocky Hill, NJ, USA). Ascorbic acid, heparin, paraformaldehyde, NaCl, Tris-HCl,
TBS, 0.1% Triton-X100, oil red, alizarin red were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA) Agarose gel was purchased from Sangon Biotechnology Co. (Shanghai, China).

Isolation and cultivation of hDPSCs

hDPSC isolation and flow cytometry analyses were carried out as described previously. Teeth were cleaned and cracked open using a vice to reveal the pulp chamber. The pulp tissue was gently separated from the crown and the root, and then digested in a solution containing 3 mg/mL collagenase type I and 4 mg/mL dispase for 1 hour at 37°C. Single-cell suspensions were obtained by passing the cells through a 70 μm Cell Filter strainer.

Medium preparation for the induction of spinal motor neuronal differentiation

The induction of spinal motor neuronal differentiation was carried out as previously described with some modifications. DPSCs were used for experiments at passage 5. Cell culture media were based on neuronal induction media 500 mL comprising DMEM/F12 489 mL containing N2 5 mL, nonessential amino acids 5 mL, and heparin 1 mL (1 mg/mL) supplements. On Day 0 of differentiation, the medium was replaced with fresh neuronal induction medium supplemented with all-trans retinoic acid at a final concentration of 0.1 μM. On Day 4, the cells were cultured in neuronal induction medium containing sonic hedgehog (SHH) (100 ng/mL) and retinoic acid (0.1 μM). On Day 6 of differentiation, the cells were treated with neuronal induction medium supplemented with retinoic acid (0.1 μM), SHH (100 ng/mL), cAMP (1 μM), and ascorbic acid (200 ng/mL). On Day 9, the attached cells were treated with neuronal induction medium supplemented with BDNF, GDNF, IGF-1 (10 ng/mL, respectively), cAMP (1 μM), and ascorbic acid (200 ng/mL). The cells could be maintained in the same medium for 9–15 days.

Medium preparation for the induction of dopaminergic neuronal differentiation

The induction of dopaminergic neuronal differentiation was carried out as previously described with some modifications. DPSCs at passage 5 were used for the experiments. Cell culture media were based on N2 media 500 mL comprising DMEM/F12 495 mL and N2-A 5 mL supplements. On Day 0 of differentiation, the medium was replaced with fresh N2 media supplemented with noggin at the final concentration of 300 ng/mL. On Day 4, the cells were cultured in BCT-GA medium containing N2 medium, BDNF 50 μg/mL, ascorbic acid 200 mM, GDNF 10 ng/μL, TGF-βIII 2 μg/mL, and cAMP 200 mM. The cells could be maintained in the same medium for 12–15 days.

XTT assay

The cell viability was determined 15 days after seeding the cells using an XTT ([2,3-bis-(2-methoxy-4-nitro-5-sulfo phenyl)-2H-tetrazolium-5-carboxanilide]) Cell Proliferation Kit (Biological Industries, Kibbutz Beit HaEmek, Israel). The procedure is based on the activity of mitochondrial dehydrogenase, which is inactivated shortly after cell death. XTT is metabolized to form a soluble formazan dye that can be colorimetrically determined at 490 nm using a spectrophotometer (Thermo Varioskan Flash; Thermo, NH, USA). After incubation for 0 days, 4 days, 9 days, 12 days, and 15 days, 50 μL of XTT reaction solution was added to each well for another 3 hours of incubation. Six parallel replicates were prepared. Controls underwent the same treatment.

Immunocytochemistry

Cells were fixed with 4% paraformaldehyde and washed in Tris-buffered saline (TBS; 0.15 M NaCl, 0.1 M Tris–HCl, pH 7.5), then blocked with a solution composed of TBS, 0.1% Triton-X100 (only for intracellular antigens), and 1% bovine serum albumin (BSA). The same solution was used during the incubations with antibodies. Primary antibodies were applied overnight at 4°C. Fluorochrome-conjugated species-specific secondary antibodies were used for immunodetection. The proteins of nestin and βIII-tubulin (βIII-tub) are found almost exclusively in neurons. GFAP is an intermediate-filament protein that is highly specific for cells of astroglial lineage. O1 is an antigen characteristic of oligodendrocyte neuronal-associated markers expressed by DPSCs cultured in different neuronal inductive conditions and determined by immunocytochemical analysis. The following antibodies and final dilutions were used. Primary antibodies were anti-NeuN, -GFAP, -nestin, and -βIII-tub. For negative controls, the primary antibodies were omitted. PBS instead of the primary antibodies was used for negative controls. Secondary antibodies were FITC-conjugated goat anti-mouse immunoglobulin G (IgG), rhodamine-conjugated goat anti-mouse IgG, FITC-conjugated goat anti-rabbit IgG, rhodamine-conjugated anti-rabbit IgG, FITC-conjugated rat anti-goat IgG, and R-Phycocerythrin (RPE)-conjugated rabbit anti-goat IgG (Millipore, Billerica, MA, USA). Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI; Sigma) at 0.25 mg/mL. All the samples were examined under a fluorescence microscope (Leica Optical, Wetzlar, Germany). To quantify the number of cells positive for dopaminergic or neuronal markers and the number of cells coexpressing both types of markers, captures of immunostained cultures derived from two different donors were obtained. Cell counts for single- or double-labeled specimens were performed on three nonoverlapping randomly selected fields in cultures. The results were expressed as mean ± standard deviation (SD).
**Table 1 Primers for PCR.**

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<tr>
<th>Target cDNA</th>
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<th>Length (bp)</th>
<th>NCBI no./refs</th>
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<tr>
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<td>Antisense 5'-GTCGTCGATAGCCACAGA-3'</td>
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<td>Osteogenic marker primer</td>
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<td>Stem cell marker primer</td>
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<td></td>
<td>Antisense 5'-CGACCATCTGCGCCTTTGAG-3'</td>
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<td>Nanog</td>
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<td>Antisense 5'-CGACCATCTGCGCCTTTGAG-3'</td>
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<td>Neuron stem cell marker primer</td>
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<td>Antisense 5'-AGCCGCATCTTTCTTTGCGT-3'</td>
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ALP = alkaline phosphatase; jII-tub = jII-tubulin; bp = base pairs; BSP = bone sialoprotein; cDNA = complementary DNA; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; LPL = lipoprotein lipase; NCBI = National Center for Biotechnology Information; PCR = polymerase chain reaction; PPAR-2 = peroxisome proliferator-activated receptor.

**RT-PCR for molecular analysis**

Total RNA was extracted from the cells with RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. The gene-specific primers listed in this experiment are described in Table 1. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. The PCR products were resolved by 1.7% agarose gel and confirmed by DNA sequencing (Sangon Biotechnology Co., Shanghai, China).

**Results**

**Culture, identification, and characterization of DPSCs**

After a single-step enzymatic treatment, viable cells with elongated shapes were detected 1 day after the onset of culturing. These cells formed colonies upon further cultures. Cells from a single colony were isolated and subcultured mechanically (1st passage). Subcultured cells gradually became flattened and acquired a fibroblast-like morphology (Fig. 1). In this study, the presented results were from one single colony. Moreover, hDPSCs were also tested to determine their expression of stem cell-specific transcription factors. The expression of adipogenic peroxisome proliferator-activated receptor 2 (PPAR2) and lipoprotein lipase (LPL), osteogenic alkaline phosphatase (ALP) and bone sialoprotein (BSP), and stem cell (Oct-4 and Nanog) markers were also detected by RT-PCR. These results confirmed that DPSCs are capable of differentiation into at least two different cell types, such as adipogenic and osteogenic cells (Fig. 2).

The expression profiles of a total of nine cell surface antigens were shown in hDPSCs (Fig. 3), and these were also compared with published profiles of hDPSCs.\textsuperscript{30} CD44, CD73, CD90, CD105, and CD166, which are common hDPSC markers, were expressed in our hDPSCs. However, CD14, CD34, and CD45, the markers specific for hematopoietic cells, were not expressed in our hDPSCs. These results indicate that our hDPSCs are comparable to the expression profiles of hDPSCs.\textsuperscript{30}

**Morphological change of DPSCs following neuronal induction**

In order to assess the efficacy of different media formulations on dopaminergic and spinal motor neuronal differentiation of hDPSCs, we used two different neuronal media to promote the neuronal differentiation of embryonic stem cells as previously described\textsuperscript{28,29} with some modifications. Morphological changes in the spindle shape of DPSCs appeared by Day 5 of differentiation and peaked by Day 15 when the majority of the cells assumed a multipolar appearance with cellular processes extending from cell bodies (Fig. 4). However, some fibroblast-like cells with spread-out morphology were still observed in the population. A small fraction of flat, round oligodendrocyte-like cells were observed; the proportion was bigger in the group plated with high cell density (data not shown). This study found that DPSCs fulfilled these criteria, because DPSCs cultured in expansion medium (DMEM + 10% FCS) expressed constitutively neuronal markers jII-tub and nestin (Fig. 4).
Proliferation potential of DPSCs

After 9 days of induction, DPSCs exposed to either dopaminergic or motor neuronal inductive media acquired neuron-like morphology. Assessment of the cell proliferation status of DPSCs over the same period showed that there was a significant 2.6- and 2-fold increase in the proliferation rate of DPSCs cultured in dopaminergic neuronal inductive media ($p < 0.002$; Student $t$ test) and spinal motor neuronal inductive media ($p < 0.004$) for 15 days.

Figure 1  Morphological changes in dental pulp stem cells (DPSCs) in cultures at early time points. Cell morphology of one representative DPSC culture at (A) Day 1, (B) Day 4, (C) Day 7, and (D) Day 14. Cultured cells gradually became flattened and acquired a fibroblast-like morphology. (A–D, magnification 20×, after plating).

Figure 2  Characterization of human dental pulp stem cells (hDPSCs). hDPSCs are spindle- and fibroblast-like cells. (A) After culturing under adipogenic conditions for 15 days, hDPSCs formed lipid droplets when stained with oil red. (B) When culturing in osteogenic medium for 15 days, hDPSCs formed mineralized nodules by alizarin red staining. (C) Expression of adipogenic (PPAR2 and LPL), osteogenic (ALP and BSP), and stem cell (Oct-4 and Nanog) markers.
days, respectively, compared with those in control cultures, as demonstrated by the XTT assay (Fig. 5).

Quantity analysis of neuronal induction of DPSCs in dopaminergic and motor neuronal inductive media

We assessed two different neuronal media conditions that were described previously to promote neuronal differentiation only in embryonic stem cells. The protein expression patterns of βIII-tub, GFAP, and O1 neuronal-associated markers expressed by DPSCs cultured in different neuronal inductive media were determined by immunocytochemical analyses (Fig. 6). The data demonstrated a progressive increase in neuron (βIII-tub), astroglial cell (GFAP), and oligodendrocyte (O1) neuronal markers after induction. The proportions of βIII-tub-, GFAP-, and O1-positive cells were significantly higher (dopaminergic neuronal media: $p < 0.05$, $p < 0.0005$, and $p < 0.05$, respectively; spinal motor neuronal media: $p < 0.0005$, $p < 0.0005$, and $p < 0.05$, respectively).

Figure 3 Characterization of the human dental pulp stem cell (hDPSC) immunophenotype in vitro.

Figure 4 Dental pulp stem cells (DPSCs) cultured in neural inductive media expressed (A,B) neuron-like morphology and (C) neurogenic [nestin and βIII-tubulin (βIII-tub)] markers that were determined by reverse transcription polymerase chain reaction (RT-PCR).
p < 0.005, and p < 0.005, respectively; Student t test) in DPSCs cultured in both neuronal inductive media than in DPSCs cultured in control media (Fig. 7). Collectively, these data suggest that in response to neuronal inductive stimuli, a greater proportion of DPSCs stopped proliferation but acquired a phenotype resembling mature neurons.

After culturing the cells in the induction media for 15 days, immunofluorescence staining identified cell populations expressing the neuronal protein βIII-tub. Expression of the dopaminergic-like cell marker TH or cholinergic motor neuron marker ChAT was also detected in these cells. Double immunostaining showed that some cells expressed βIII-tub and TH or ChAT. The percentages of the

Figure 5 The proliferation potential of dental pulp stem cells (DPSCs) following the neuronal differentiation assay is represented as absorbance readings after an XTT [(2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide)] assay of cells cultured in the corresponding media. When DPSCs were cultured in dopaminergic or motor neuronal inductive media for 15 days, there was a 2.6- or 2-fold decrease in proliferation potential, respectively.

Figure 6 (A–C) The induction of dopaminergic neural differentiation of dental pulp stem cells (DPSCs). Immunocytochemical staining of cultured DPSCs may express a variety of neuronal markers including βIII-tubulin (βIII-tub), glial fibrillary acidic protein (GFAP), and O1 (red) when cultured in dopaminergic neurogenic media for 15 days. (D–F) The induction of spinal motor neural differentiation of DPSCs. Immunocytochemical staining of cultured DPSCs may express a variety of neuronal markers including βIII-tub, GFAP, and oligodendrocyte (O1; green) when cultured in spinal motor neural media for 15 days. The blue fluorescence is the cell nucleus. Scale bars = 400 μm.

Figure 7 The number of dental pulp stem cells (DPSCs) positively stained for the respective neuronal-specific antibodies represented as a percentage of field of view. An increase in βIII-tubulin (βIII-tub), glial fibrillary acidic protein (GFAP), and O1 (red) staining was found in DPSCs cultured in neuronal inductive conditions when compared with the control. Quantitative analyses of βIII-tub, GFAP, or O1 expression. From 50 to 100 cells per group were analyzed in randomly chosen fields. Data are mean ± standard deviation (SD) of triplicate cultures. *p < 0.05, **p < 0.005, and ***p < 0.0005; Student t test.
Figure 8  Human dental pulp stem cell (hDPSC)-derived dopaminergic cells expressing the neuronal markers βIII-tubulin (βIII-tub) and the dopamine neuron marker tyrosine hydroxylase (TH) 15 days after induced differentiation [the cell nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI) in A–D]. hDPSC-derived spinal motor neural cells expressing the neuronal markers βIII-tub and cholinergic neuron marker choline acetyltransferase (ChAT) 15 days after induced differentiation (the cell nuclei were counterstained with DAPI in E–F). Scale bars = 200 μm. The percentages of βIII-tub- and TH-positive cells in hDPSC-derived dopaminergic cells and the percentages of βIII-tub- and ChAT-positive cells in hDPSC-derived spinal motor neuron cells after induction were significantly higher than those before induction. Data are mean ± standard deviation (SD) of triplicate cultures. *p < 0.005 and **p < 0.0005; Student t test.

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positive cells for βIII-tub and TH or ChAT are summarized in Fig. 8. The data revealed progressive enhancement in βIII-tub/TH (8.3 ± 2.58% vs. 27.3 ± 6.97%, p < 0.005) and βIII-tub/ChAT (10.2 ± 2.14% vs. 52.8 ± 4.26%, p < 0.005). When DPSCs were cultured in neuronal inductive media, the protein level of the neuronal markers βIII-tub/TH or βIII-tub/ChAT appeared to be unregulated. Collectively, these results suggest that in response to the neuronal inductive stimuli, DPSCs acquired a phenotype resembling neuron-like cells.

Discussion

Transplanted human MSC (hMSC) may alter the tissue microenvironment in a way that promotes survival of endogenous cells, including injured neurons, immature oligodendrocytes, and oligodendrocyte progenitor cells. Thus, hMSCs cultured with neuronal inductive media may transdifferentiate into neuron-like cells for nerve regeneration.

Dopaminergic neuronal inductive media and motor neuronal inductive media have been completely investigated in hMSCs or BMSCs. Although the direct effect of BMSC transplantation may be for cells to differentiate into neuron-like cells, endothelial cells, and astrocytes, these cells still have limited survival and differentiation. To date, as we can see from searching the literature, there has been no study on the effect of dopaminergic and motor neuronal inductive media on DPSCs. Because DPSCs originate from the cranial neural crest and have neural characteristics, we suggest culturing DPSCs in dopaminergic and motor neuronal inductive media in order to obtain neuron-like cells for therapeutic use. Investigations on the expression of neuron-specific cell markers in DPSCs by immunocytochemistry and quantitative RT-PCR revealed that such inductive media may generate more completely-developed neuron-like cells from hDPSCs.

This study showed that our DPSCs were also capable of differentiation into adipocytes and osteogenic cells. Our results are similar to the previous findings that DPSCs are capable of differentiating into at least two different cell types, which are normally derived from different embryonic germ layers.

In the present study, differentiated cells began to divide slowly after 24 hours of culture and continued to proliferate until the end of the differentiation period, suggesting that the cells underwent differentiation immediately after neuronal differentiation had been induced. Although the number of cells increased upon the induction of differentiation, the cells subsequently showed a reduction in growth rate and focused on the differentiation process. In this study, there was a decrease in DPSCs cultured in dopaminergic or motor neuronal inductive media, respectively, as revealed by the XTT assay.

We suggest that DPSCs changed gradually and differentiated into neuron-like cells after 7 days when cultured in serum-free medium, because changes that occurred in cell microenvironments such as removal of serum and addition of certain growth or differentiation factors were found to be able to induce transdifferentiation of DPSCs in vitro. This study also found that DPSCs may transdifferentiate into neuron-like cells when serum is absent during culturing.

The proportions of βIII-tub, GFAP, and oligodendrocyte (O1)-positive cells were significantly higher in DPSCs cultured in both inductive media than in DPSCs cultured in the control media. The high expression of both nestin and βIII-tub in DPSCs cultured in both inductive media indicates that DPSCs can differentiate into neuron-like cells in both inductive media. Nestin is known to be expressed within fibrous dental pulp tissue. However, its expression continued to be detected by the majority of DPSCs following neuronal induction; that is, expression of nestin was increased when cells differentiated into neurons. βIII-tub is expressed only after neuronal differentiation and is therefore utilized as a marker of mature neuron cells during the final stages of growth. Furthermore, hDPSC-derived dopaminergic and spinal motor neuron-like cells expressed significantly higher neuronal markers than prior to induction. These findings suggest that in response to the neuronal inductive stimuli, a greater proportion of DPSCs stopped proliferation and acquired a phenotype resembling mature neurons.

Stem cells can be used for tissue repair, treatment of disease, and drug toxicity testing. The results of this study support the use of DPSCs as promising candidates for cell-based therapies for neuron disorders. DPSCs are easily available from adult teeth such as wisdom teeth that are sometimes extracted due to malpositioning. This procedure of preparation of DPSCs is devoid of any relevant ethical concerns. In addition, DPSCs can be easily handled in the laboratory. They are expandable, cryopreservable, and robust in culture. However, future studies should be carried out to clarify whether DPSCs, apart from their neurotrophic and neuroprotective effects, are also able to differentiate into fully functional neurons.

Acknowledgments

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