Isolation and Characterization of Human Dental Pulp Stem/Stromal Cells From Nonextracted Crown-fractured Teeth Requiring Root Canal Therapy

Anderson Hsien-Cheng Huang, MS,[*†#] *Yuk-Kwan Chen, MS,*[*‡#] *Anthony Wing-Sang Chan, PhD,*[¶] *Tien-Yu Shieh, PhD,*[§‖] *and Li-Min Lin, PhD*[*‡]

Abstract

Introduction: Human dental pulp stem/stromal cells (hDPSCs) in adults are primarily derived from the pulp tissues of permanent third molar teeth in existing literatures, whereas no reports exist, to our knowledge, on deriving hDPSCs from a tooth without the need for surgical procedure. The aim of this study was to raise a novel idea to source hDPSCs from complicated crown-fractured teeth requiring root canal therapy. **Methods:** hDPSCs were harvested from the pulp tissues for two complicated crown-fractured teeth requiring root canal therapy, retaining the teeth for subsequent prosthodontic rehabilitation, in a 41-year-old woman who had suffered a motorcycle accident. Pulp tissue from the left lower deciduous canine of a healthy 10-year-old boy (the positive control) was also removed because of high mobility and cultured for hDPSCs. **Results:** The hDPSCs derived from the two complicated crown-fractured teeth and the deciduous tooth were able to differentiate into adipogenic, chondrogenic, and osteogenic lineages and also expressed stem cells markers and differentiation markers, which indicated their stem cell origin and differentiation capability. In addition, hDPSCs from both the complicated crown-fractured teeth and the deciduous tooth showed high expression for bone marrow stem cell markers including CD29, CD90, and CD105 and exhibited very low expression of markers specific for hematopoietic cells such as CD14, CD34, and CD45. **Conclusions:** This report describes the successful isolation and characterization of hDPSCs from the pulp tissue of complicated crown-fractured teeth without tooth extraction. Therefore, pulp exposed in complicated crown-fractured teeth might represent a valuable source of personal hDPSCs. *(J Endod 2009;35:673–681)*

Key Words

Crown fracture, dental pulp, dental pulp stem cell

Human dental pulp stem/stromal cells (hDPSCs) possess gene expression profiles and a differentiation capacity analogous to that of bone marrow–derived stem cells (BMSCs) (1, 2). hDPSCs are potentially more accessible and may be easier to obtain compared with adult bone marrow stem cells after pulp exposure and endodontic treatment. Most recently, there are many reports discussing the pulp tissue engineering with stem cells from human dental pulp (3–9). Therefore, banked hDPSCs may provide a good prospective in future regenerative dental and medical treatment that may involve controlled direct employment of the cells in situ and possible seeding of stem cells at areas of injury for regeneration or use of the stem cells with appropriate scaffolds for tissue engineering solutions (3, 6).

Previous research on hDPSCs has principally relied on pulp tissues derived from healthy primary incisors and permanent third molar teeth (10). Most recently, our laboratory has been successful in isolating and characterizing hDPSCs from a mesiodens (11). Trauma to the oral and maxillofacial region sustained during the course of routine daily activities (12). The reported incidence and prevalence of traumatic injuries to teeth are variable, depending on the literature sources; the most affected dental location is the maxillary incisors (13). To our knowledge, there has been no detailed study of the derivation of hDPSCs from a permanent tooth that does not involve invasive surgical procedure. In this article, we report the successful isolation and characterization of hDPSCs from the pulp tissues of two complicated crown-fractured teeth that required endodontic treatment in order to retain the teeth for subsequent prosthodontic rehabilitation, thereby extending the potential source of hDPSCs.

Materials and Methods

Isolation and Culture of hDPSCs

A 41-year-old woman suffered complicated crown fractures with pulp exposure of the left upper central and lateral incisors because of a motorcycle injury (Fig. 1*A* and *B*). The dental pulp tissues of both complicated crown-fractured teeth were extracted 4 days later using an endodontic file, with the informed consent of the patient (Fig. 1*C*) and institutional review board approval from our institution (KMUH-IRB-980016). After endodontic treatment (Fig. 1*D*), porcelain crowns for each of the two crown-fractured teeth were fabricated (Fig. 1*E*).

A deciduous tooth (left lower canine) was also removed from a healthy 10-year-old boy because of high mobility and used as a positive control; removal was performed

From the *Department of Oral Pathology, College of Dental Medicine, Kaohsiung Medical University, Taiwan; †PhD Program, School of Dentistry, College of Dental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; ‡Division of Dentistry, Department of Oral Pathology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; ¶Yerkes National Primate Research Center, Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, USA; §Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, College of Dental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; and ‖Division of Dentistry, Department of Oral and Maxillofacial Surgery, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan.

#Anderson Hsien-Cheng Huang and Yuk-Kwan Chen contributed equally to this manuscript.

Address requests for reprints to Dr Yuk-Kwan Chen & Dr. Li-Min Lin, Department of Oral Pathology, School of Dentistry, Kaohsiung Medical University, Kaohsiung Medical University Hospital, 100 Shih-Chuan 1st Road, Kaohsiung, Taiwan. E-mail address: k0285@ms22.hinet.net.

0099-2399/$0 - see front matter

doi:10.1016/j.joen.2009.01.019

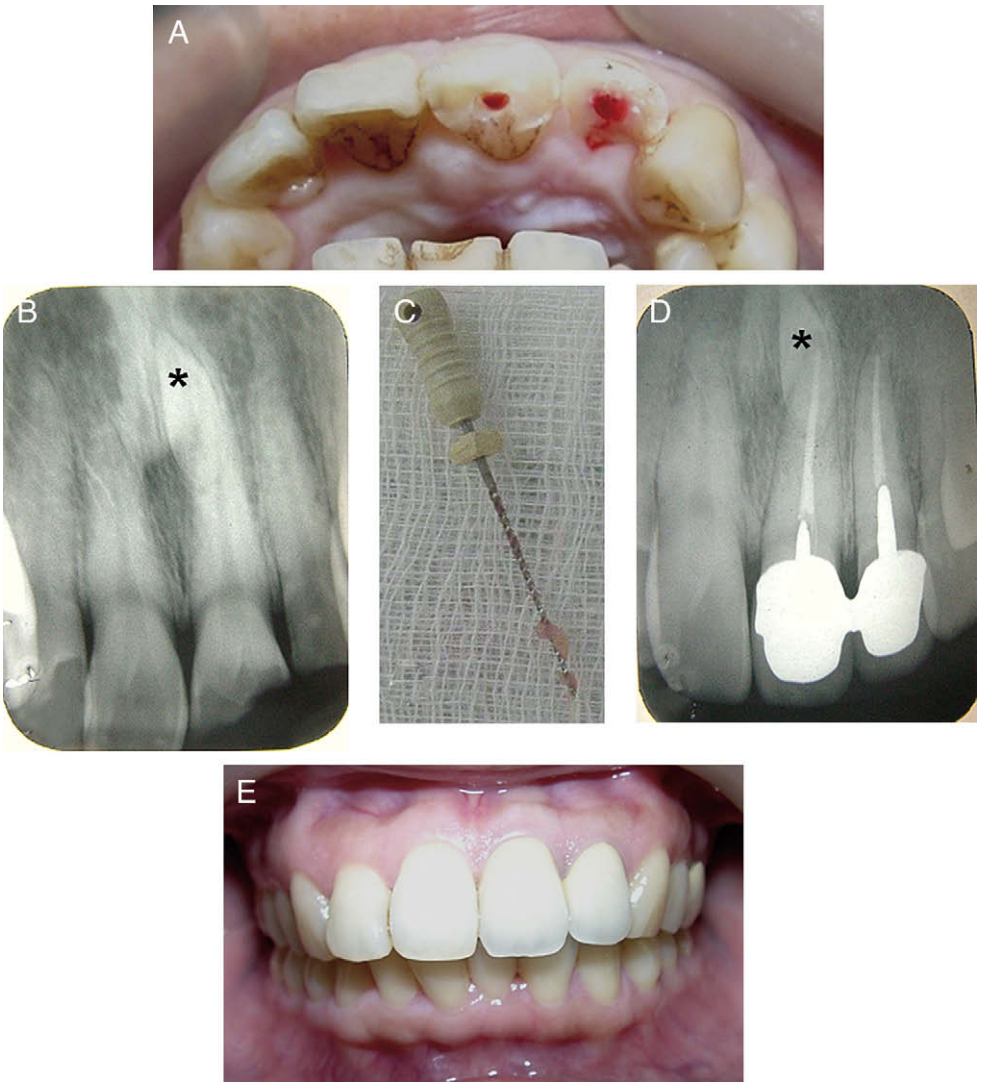

Figure 1. A (*A*) clinical and (*B*) periapical radiograph of the two complicated crown fractures with pulp exposure of the left upper central and lateral incisors. The dental pulp tissues of both complicated crown-fractured teeth were extracted using an endodontic file (*C*). After endodontic treatment (D), porcelain fused to metal crowns for each of the two crown-fractured teeth was fabricated (E) (Asterisk indicates that a mesiodens is noted on the periapical radiograph).

under local anesthesia after the consent of the patient's parents with institutional review board approval from our institution (KMUH-IRB-980016). The surface of the deciduous tooth was first cleaned with methanol (70% alcohol). The deciduous tooth was then kept on ice in Dulbecco's phosphate-buffered saline (DPBS; Invitrogen, Carlsbad, CA) and delivered to the laboratory for the isolation of hDPSCs. A groove of 0.5 to 1.0 mm in depth was cut around the circumference of the tooth using a sterile handheld high-speed drill. The dental pulp was then exposed by splitting the tooth with a chisel along the groove, and the pulp tissue was extracted using an endodontic file.

The extracted pulp tissues of the two complicated crown-fractured teeth and the deciduous tooth were then digested in collagenase type I (3 mg/mL, Invitrogen) and dispase (4mg/ml, Invitrogen) for 1 hour at 37°C (11). The resulting cell suspensions were filtered through a 40-μm cell strainer (Falcon, BD, Franklin Lakes, NJ), and the single cell suspensions were then cultured in a DPSC medium containing α-MEM (Hyclone, Logan, UT), 20% fetal bovine serum (Hyclone), 100 μmol/L L-ascorbic acid-2-phosphate (Sigma, St Louis, MO), 100 U/mL penicillin (Invitrogen), and 100 μg/mL streptomycin (Invitrogen). The hDPSCs derived from the two complicated crown-fractured teeth and the deciduous tooth were cultured at 37°C with 5% CO_2; the medium was refreshed every 2 days, and 80% confluence was obtained for optimal cell harvesting.

Proliferation Rate of hDPSCs

The proliferation rates of the hDPSCs obtained from the complicated crown-fractured teeth and the deciduous tooth were determined by plating 25,000 hDPSCs (passage 3) per well on a six-well plate; each had three replicas and was passaged and counted at every 72 hours. Cells were counted before the next passage, and 25,000 cells were replated for a total of four passages. The proliferation rate was then calculated by dividing the total cell number after every 72 hours of culture by the initial plating number (25,000).

Data analyses for the differences in different tooth groups and different proliferation rates on passages were performed by a one-way analysis of variance in Statistical Analysis Systems (version 14; SPSS, Chicago, IL).

Colony-forming Unit Assay

The 3-passage hDPSCs from both the complicated crown-fractured teeth and the deciduous tooth were seeded in a six-well plate at

TABLE 1. Oligoprimers Targeting Stem Cell and Differentiation Markers, and GAPDH, Which Was Used as the Control

Oligoprimers	Sequences	PCR products
Oct4	Forward: 5′-GAC AAC AAT GAA AAT CTT CAG GAG A-3′ Reverse: 5′-TAC AGA ACC ACA CTC GGA CCA-3′	205bp
Rex-1	Forward: 5′-AGA ATT CGC TTG AGT ATT CTG A-3′ Reverse:5′-GGC TTT CAG GTT ATT TGA CTG A-3′	470bp
Nanog	Forward: 5′-TCT CTC CTC TTC CTT CCT CCA-3′ Reverse: 5′-GGA AGA GTA GAG GCT GGG GT-3′	389bp
Nestin	Forward: 5′-TGG CAA GGC GAC TGG GCG A-3′ Reverse: 5′-CCC TCT ATG GCT GTT TCT TTC TCT-3′	810bp
Osteonection	Forward: 5′-ATC TTC TTT CTC CTT TGC CTG G-3′ Reverse: 5′-GCA CAC CTC TCA AAC TCG CC-3′	323bp
GAPDH	Forward: 5′-ATG GGG AAG GTG AAG GTC GG-3′ Reverse: 5′-CCA TCA CGC CAC AGT TTC CC-3′	596bp

40 single cells per well with three replicas. After 2 weeks in culture, the cells were fixed in 10% buffered formalin for 10 minutes and stained with 3% (v/v) crystal violet (Sigma) for 5 minutes; they were then washed twice with distilled water and the number of colonies enumerated. Only colonies larger than 2 mm in diameter were counted: those any smaller could be fall-off cells or satellite cells that were 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 multiplying by 100 (11).

Reverse–transcriptase Polymerase Chain Reaction

To determine whether the hDPSCs of the complicated crown-fractured teeth and the deciduous tooth shared similar expression patterns of stem cells and differentiation markers, as has been previously described for hDPSCs (1) and BMSCs (2), reverse-transcriptase polymerase chain reaction (RT-PCR) was performed using a DNA thermal cycler (TaKaRa MP, Tokyo, Japan). First, total RNA was obtained using an RNeasy Plant Mini Kit (QIAGEN, Valencia, CA) followed by reverse transcription of messenger RNA according to the procedure provided by SuperScript III (Invitrogen). The resulting complementary DNA was subsequently used for PCR amplification. The PCR reactions were performed using specific oligoprimer sets (11), which were designed with reference to the published complementary DNA sequences in GenBank (Bethesda, MD). The amplification cycle consisted of 3 minutes at 94°C, 25 seconds at 94°C, 30 seconds at 62°C, and 50 seconds at 72°C repeated 40 times, followed by 3 minutes at 72°C. Five oligoprimer sets were designed in order to determine the expression of stem cell markers (Oct4, Rex-1, and Nanog; Table 1) and differentiation markers (Nestin and Osteonectin, Table 1). All oligoprimers were designed to cross the junction between two exons with the forward primer located at the 3′ region of the leading exon, whereas the reverse primer was located at the 5′ region of the following exon in order to minimize the possibility of a false-positive or contamination of genomic DNA. In addition, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the positive control (Table 1) and H_2O as the negative control.

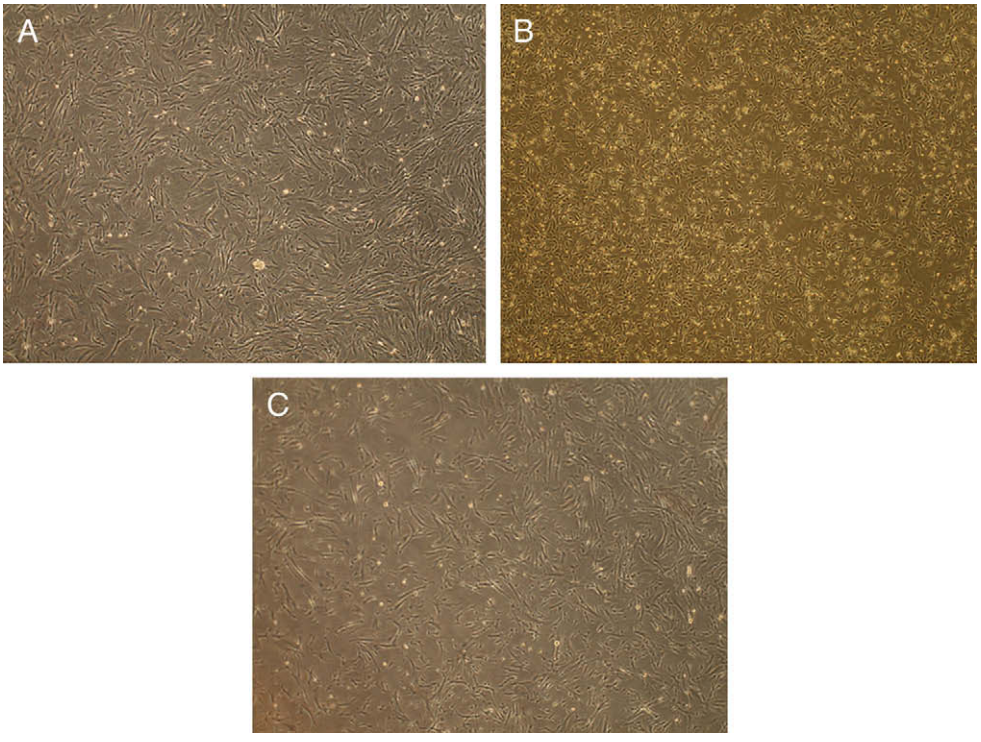

Figure 2. The human DPSCs obtained from both the complicated crown-fractured teeth (*A*, central incisor; *B*, lateral incisor) and (*C*) the deciduous tooth were spindle shaped in morphology (×100).

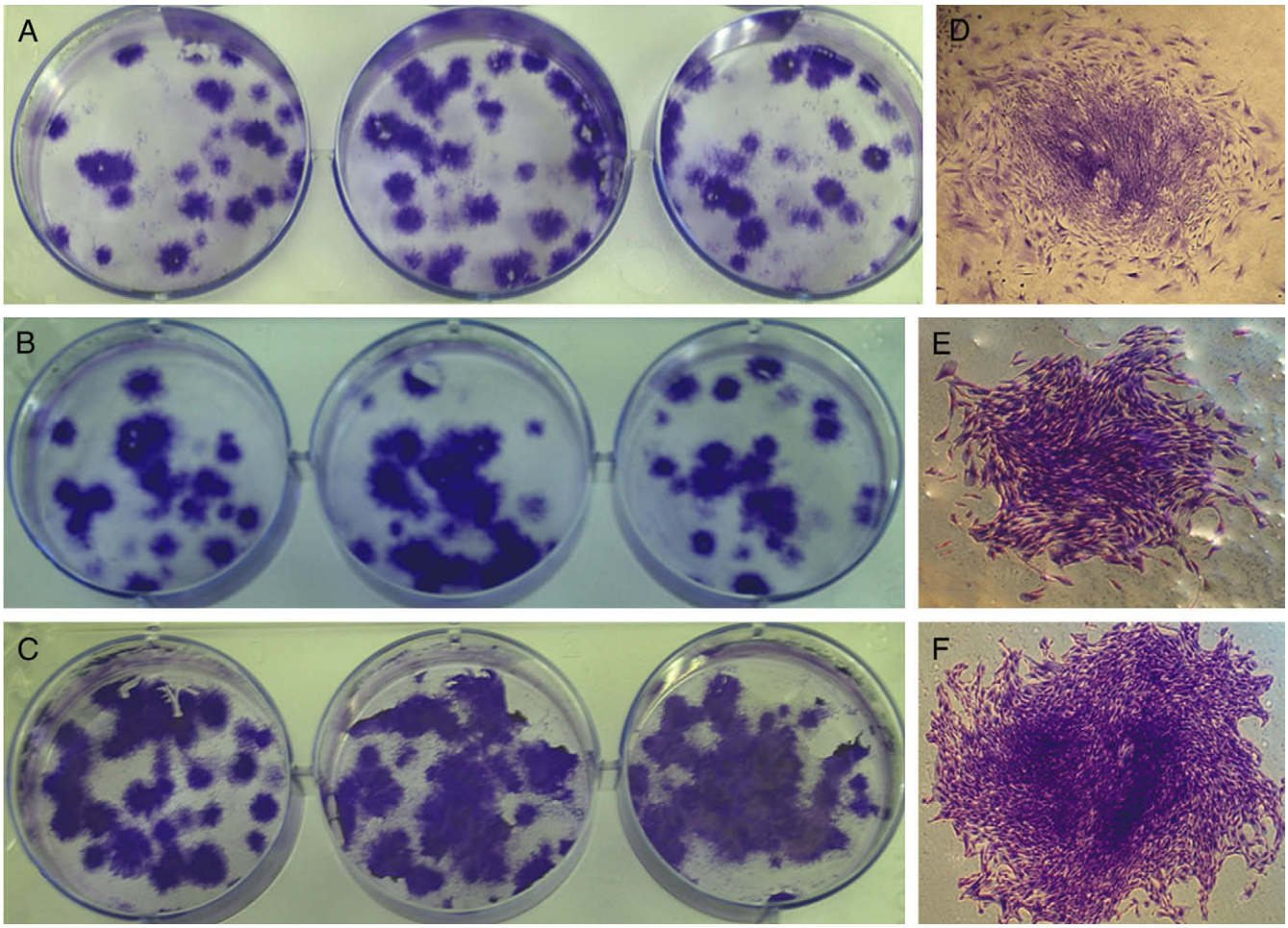

Figure 3. The human DPSCs obtained from both the complicated crown-fractured teeth (*A* and *D* [×40]: central incisor; *B* and *E* (×100): lateral incisor] and the deciduous tooth (*C* and *F* [×100]) were able to form colonies; they were stained with crystal violet.

Differentiation of hDPSCs

Adipogenic differentiation. To differentiate toward adipogenic lineage, the three-passage hDPSCs of both the complicated crown-fractured teeth and the deciduous tooth were seeded at 400 cells/cm^2 in a 35-mm tissue culture dish and cultured for 11 days in DPSC medium until confluence was achieved. The DPSC medium was subsequently supplemented with 5 μg/mL insulin (Sigma), 50 μmol/L indomethacin (Sigma), 1 μmol/L dexamethasone (Sigma), and 0.5 μmol/L isobutyl-1-methyl xanthine (Sigma) and the cultures placed in an incubator at 37°C and 5% CO_2 for another 21 days, with medium changes three times per week. As a control, DPSC medium only was used. At the end of the cultivation period, the cells were then fixed in 10% buffered formalin for 10 minutes and stained with Oil-Red-O (Sigma) to stain the lipid vacuoles (11). Furthermore, RT-PCR of PPARγ messenger RNA indicating adipogenic lineage for the hDPSCs (3-passage) of both the complicated crown-fractured teeth and the deciduous tooth were performed. Briefly, PCR conditions were 60.5°C annealing with forward oligoprimer (5′-CAG TGG GGA TGT CTC ATA A-3′) and reverse oligoprimer (5′-CTT TTG GCA TAC TCT GTG AT-3′) for 35 cycles producing a PCR product of 391 bp. These primers can detect both PPARγ-1 and -2 (14). GAPDH was used as the positive control and H_2O as the negative control (Table 1).

Chondrogenic differentiation. A total of 2.5 × 10^5 hDPSCs from both the complicated crown-fractured teeth and the deciduous tooth were centrifuged in a 15-mL conical tube at 1,000 rpm for 5 minutes. The pellet was maintained in DPSC medium supplemented with 10 μg/mL ITS-X (Invitrogen), 5.35 μg/mL linoleic acid, 1.25 μg/mL bovine serum albumin (BSA), 1.0 μg/mL dexamethasone, and 10 ng/mL TGF-β3 (Chemicon, Billerica, MA). The medium was replaced every three to four days for four weeks. As a control, DPSC medium only was used. The pellets were then fixed in 4% paraformaldehyde (PFA) PFA overnight, and paraffin-embedded sections (4-5μm) were stained with Alcian blue (15).

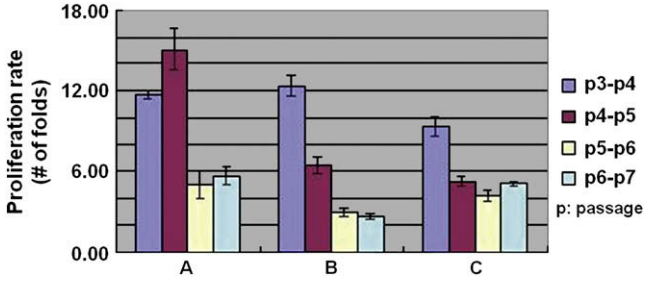

Figure 4. The proliferation rates for the complicated crown-fractured teeth (*A*: central incisor and *B*: lateral incisor) and (*C*) the deciduous tooth.

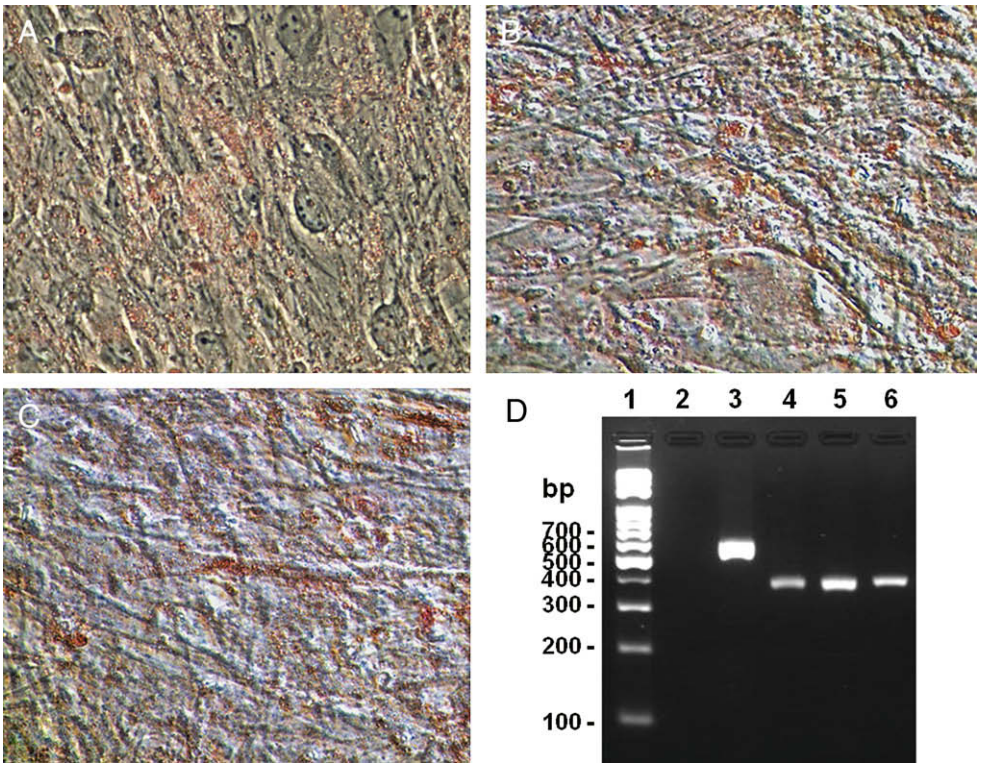

Figure 5. The human DPSCs of both the complicated crown-fractured teeth (*A*: central incisor and *B*: lateral incisor) and (*C*) the deciduous tooth were able to differentiate toward adipogenic lineage (×400). (*D*) The expression of PPARγ mRNA (391 bp) on RT-PCR also indicates adipogenic lineage of the two crown-fractured teeth (*lane 4*: central incisor; *lane 5*: lateral incisor) and deciduous tooth (*lane 6*); *lane 1*: marker; *lane 2*: H_2O (negative control); *lane 3*: GAPDH (positive control, 596 bp).

Osteogenic differentiation. The hDPSCs from the two complicated crown-fractured teeth and the deciduous tooth were prepared as described previously, with the exception that 1 nmol/L dexamethasone (Sigma), 20 mmol/L β-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 37°C and 5% CO_2 for another 21 days, with medium changes three times per week. As a control, DPSC medium only was used. At the end of the cultivation period, the DPSCs of both the crown-fractured teeth and the deciduous tooth were fixed in 10% buffered formalin for 10 minutes and stained with alizarin red S (Wako, Osaka, Japan) for 15 minutes at room temperature so that the mineral matrix of the bone could be observed (11).

Flow Cytometry

DPSCs of both the complicated crown-fractured teeth and the deciduous tooth were divided into 6 fluorescence-activated cell sorting round-bottom tubes (Becton Dickinson Falcon, Sunnyvale, CA) at 2 × 10^5cells/tube and stained with immunoglobulin G–fluorescein isothiocyanate –-conjugated or phycoerythrin-conjugated anti-CD14, -CD29, -CD34, -CD45, -CD90, -CD105 (Beckman Coulter, Villepinte, France, 20 μL each). After a 20-minute incubation at ambient temperature in the dark, cells were washed twice with 2 mL fluorescence-activated cell sorting wash solution of phosphate-buffered saline containing 0.1% phosphate buffered solution (PBS) FBS and 0.1% NaN_3 and centrifuged 5 minutes at 230*g*. Supernatant was removed, and cells were fixed with 1% formaldehyde (in phosphate-buffered saline). Respective immunoglobulin G isotype-matched controls (Beckman Coulter) were used as negative controls (16). All data were acquired by using a Coulter Epics XL (Beckman Coulter) and analyzed using EXPO32 ADC software (Beckman Coulter) and WinMDI version 2.8 (Windows Multiple Document Interafce for Flow Cytometry; freeware developed by Joe Trotter, downloadable at methods.info/software/flow/winmdi.html).

Results

The hDPSCs from both the complicated crown-fractured teeth and the deciduous tooth proliferated rapidly, with a spindle-shaped morphology (Fig. 2*A-C*). After the third passage, homogenous populations of hDPSCs of both the crown-fractured teeth and the deciduous tooth were obtained and maintained in culture based on the cell morphology. We found that on average 80% of the hDPSCs derived from the two complicated crown-fractured teeth and 83% of the hDPSCs from the deciduous tooth were capable of forming colonies (Fig. 3*A–C*).

In addition, the hDPSCs from both the complicated crown-fractured teeth and the deciduous tooth had high proliferation rates during the early passages, but the rate decreased gradually in culture. At passage 3, hDPSCs of both the complicated crown-fractured teeth proliferated at 11.70 ± 0.26 folds and 12.37 ± 0.71 folds, respectively, and by passage 7 the rate was decreased to 5.67 ± 0.71 folds and 2.70 ± 0.17 folds, respectively (Fig. 4*A* and *B*). Moreover, at passage 3, hDPSCs of the deciduous tooth proliferated at 9.33 ± 0.76 folds, and by passage 7 the rate was declined to 5.07 ± 0.15 folds (Fig. 4*C*). In different tooth groups, the analysis of variance result is nonsignificant ($p = 0.442$); however, the differences in proliferation rates on different passages, the test of homogeneity of variances, was significant ($p = 0.016$). In addition, using the Welch analysis of variance to analyze the proliferation rates on different passages indicated a significant difference ($p = 0.018$). Then, the post hoc comparison (Tamhane

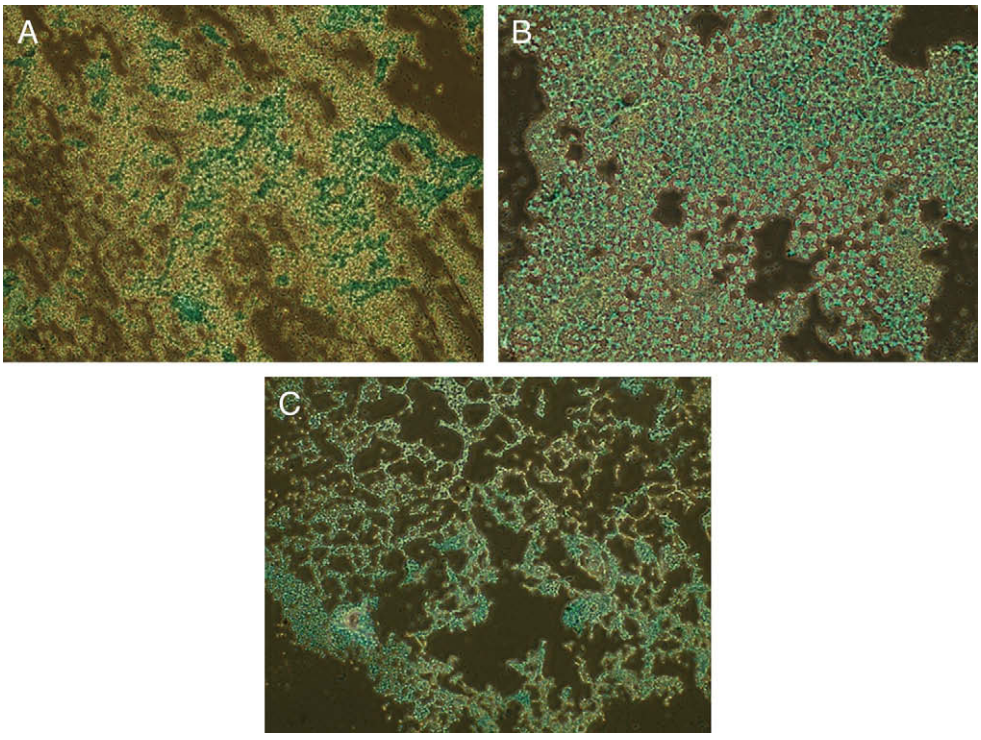

Figure 6. The human DPSCs of both the complicated crown-fractured teeth (*A*: central incisor and *B*: lateral incisor) and (*C*) the deciduous tooth were able to differentiate toward chondrogenic lineage (×100).

test) indicated significant differences among passage 3 to 4 versus passage 5 to6 and passage 3 to 4 vs passage 6 to 7.

Furthermore, the hDPSCs of both the complicated crown-fractured teeth and the deciduous tooth were able to differentiate into adipogenic (Fig. 5*A-D*), chondrogenic (Fig. 6*A–C*), and osteogenic (Fig. 7*A–C*) lineages. We also determined that the hDPSCs of the two complicated crown-fractured teeth and the deciduous tooth expressed stem cells and differentiation markers (Fig. 8*A–C*). In addition, hDPSCs

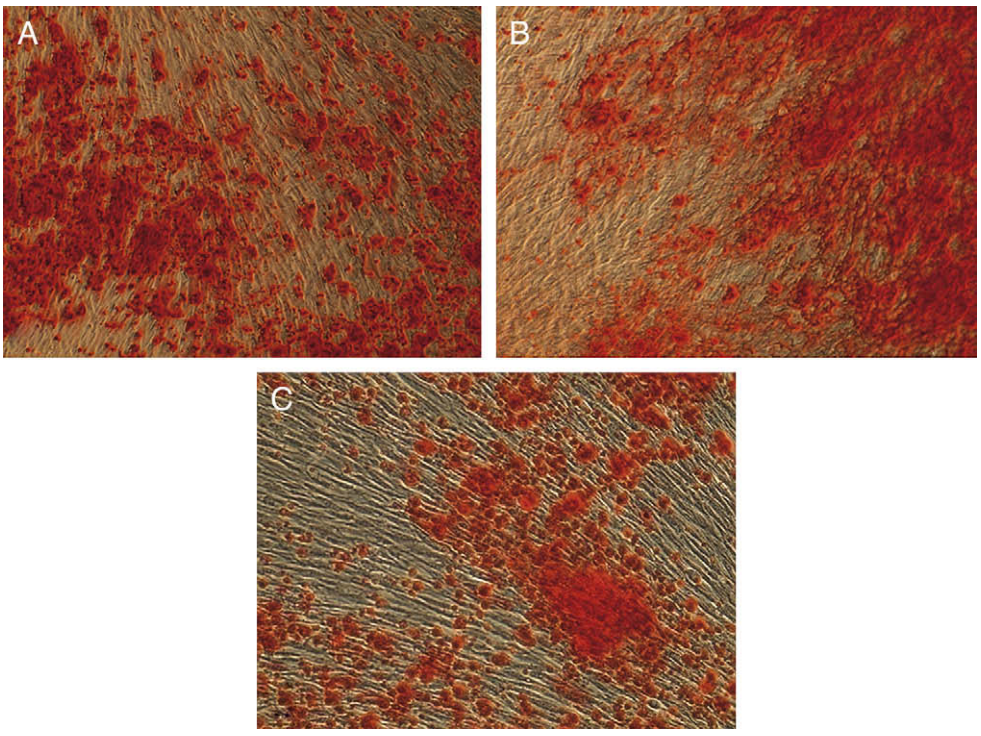

Figure 7. The human DPSCs of both the complicated crown-fractured teeth (*A*: central incisor and *B*: lateral incisor) and (*C*) the deciduous tooth (C) were able to differentiate toward osteogenic lineage (×100).

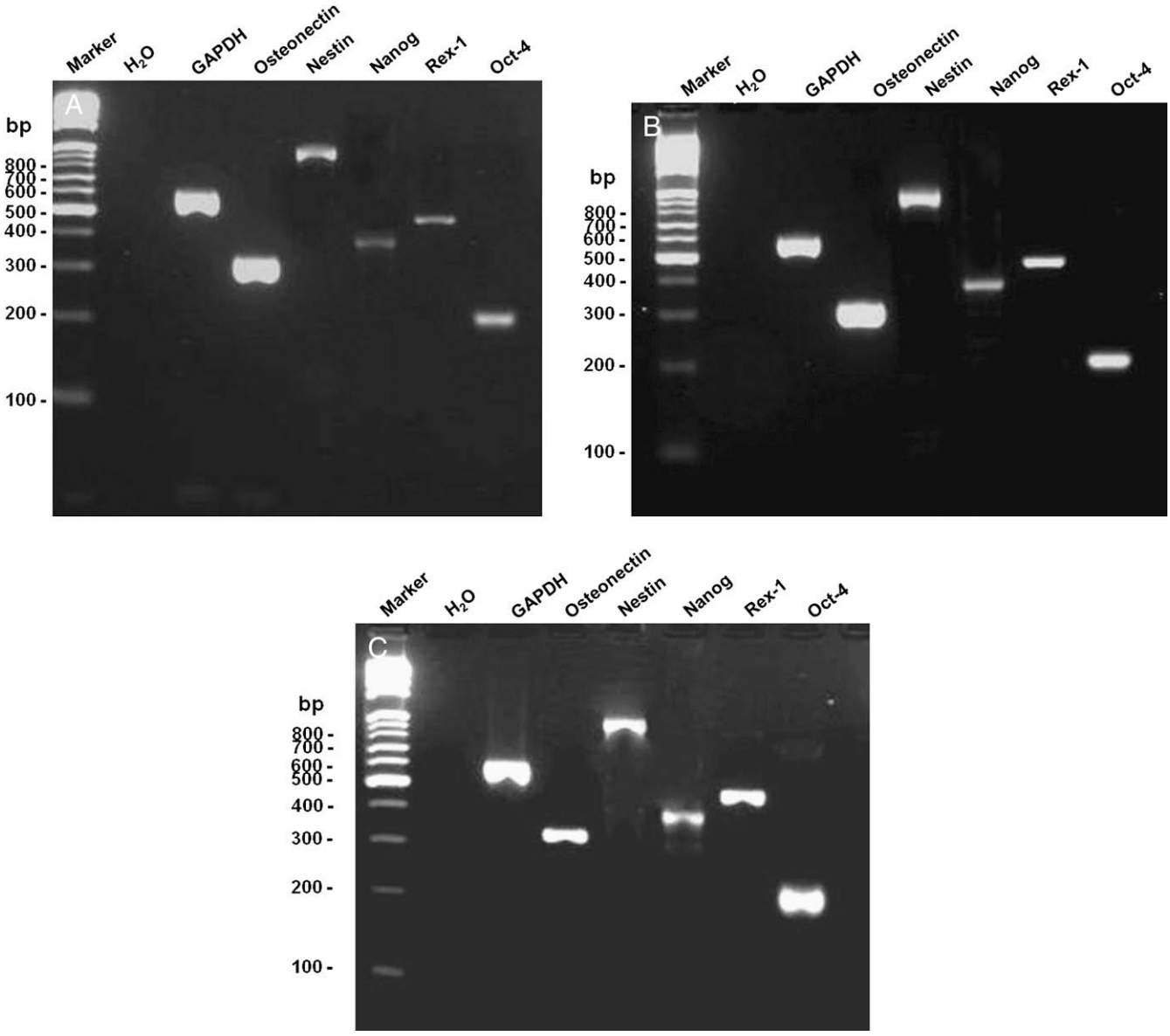

Figure 8. The human DPSCs of both the complicated crown-fractured teeth (*A*: central incisor and *B*: lateral incisor) and (*C*) the deciduous tooth expressed the differentiation (Osteonectin: 323 bp and Nestin: 810 bp) and stem cell (Nanog: 389 bp, Rex-1: 470 bp, and Oct-4: 205 bp) markers on RT-PCR. GAPDH (596 bp) was the positive control; H_2O was the negative control.

from both the complicated crown-fractured teeth and the deciduous tooth showed high expression for common BMSC markers including CD29, CD90, and CD105 (Fig. 9*A-C*). However, markers specific for hematopoietic cells such as CD14, CD34, and CD45 revealed very low expression (Fig. 9*A-C*).

Discussion

To date, three kinds of human mesenchymal stem cells have been derived from teeth: DPSCs, mostly from primary incisors and permanent third molar teeth (1); stem cells from exfoliated deciduous teeth (SHED) (17); and stem cells from immature apical papilla (18). Obtaining stem cells from immature apical papilla, although feasible, is a rather uncommon dental practice, whereas SHED has the main drawback of being a limited source because, despite the occasional retained deciduous tooth, nearly all primary teeth are exfoliated during adulthood. hDPSCs are relatively convenient stem cell resources resulting from pulp exposure and endodontic treatment. Not only can they be obtained from primary incisors and permanent third molar teeth in existing literatures, but also, as aforementioned, we extended the source of hDPSCs in our previous study by successfully isolating and characterizing hDPSCs from a supernumerary tooth (11). The incidence of supernumerary teeth varies between 0.45% and 3% (19, 20). In the current report, we have further shown the feasibility of deriving hDPSCs from a complicated crown-fractured tooth with exposed pulp. In addition, we have shown that these hDPSCs possess the adipogenic, chondrogenic, and osteogenic differentiation capacity as well as show the stem cell markers. Thus, their expression profiles and differentiation capabilities are similar to those of BMSCs (1, 2). These findings may enhance the development of a novel biologically based new generation of clinical materials for regenerative dental and medical treatments (3, 6). Significantly, the ability to isolate hDPSCs from a pulp-exposed crown-fractured tooth without requiring extraction, as shown in the

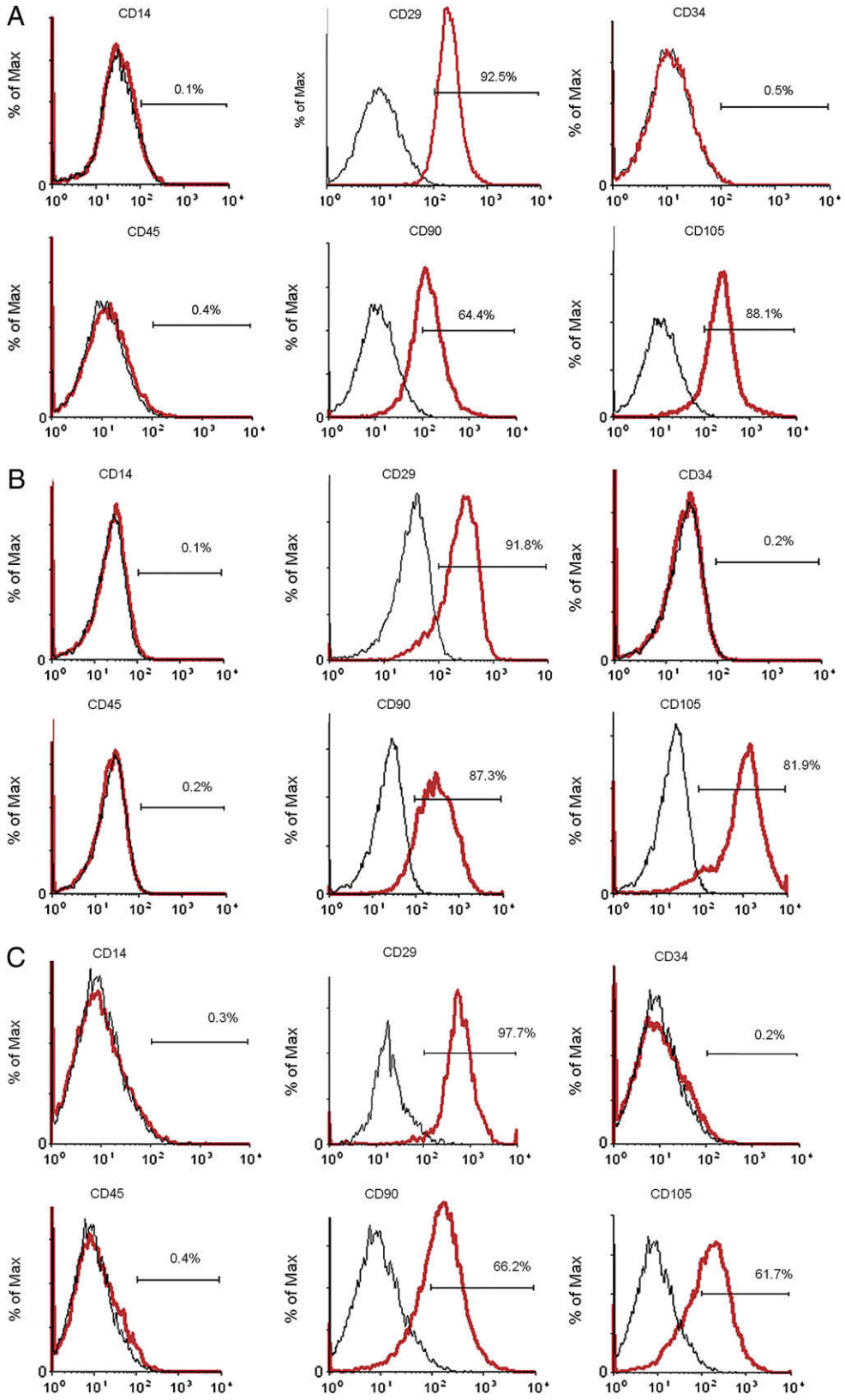

Figure 9. The human DPSCs from both the complicated crown-fractured teeth (*A*: central incisor and *B*: lateral incisor) and (*C*) the deciduous tooth showed high expression for surface markers CD29, CD90, and CD105 but very low expression for CD14, CD34, and CD45 (black line, isotype control; red line, marker of interest; Max, maximum).

present study, further extends the potential source and possibilities for hDPSCs. The incidence of complicated crown fractures with pulp exposure ranges from 4.6% to 24.4% (20–22); moreover, hDPSC acquisition from this source has the additional advantage that hDPSCs can be obtained without invasive surgical procedures. It is, of course, unethical to perform an experiment to obtain hDPSCs from a nondiseased vital tooth; however, in line with and further expanding on the findings presented in this report, it is at least theoretically indicated that, in the future, with the progress of regenerative dental and medical treatments, a person could opt to have his/her own hDPSCs removed from his/her own tooth whenever necessary for regenerative dental and medical treatments without the necessity of tooth extraction, which indicates an immense resource of personal hDPSCs.

In conclusion, this report describes the successful isolation and characterization of hDPSCs from the pulp tissue of complicated crown-fractured teeth without the need for tooth extraction. Therefore, pulp exposed in complicated crown-fractured teeth might represent a valuable source of hDPSCs.

Acknowledgments

We wish to acknowledge the technical assistance of S.M. Ji, J.Y. Shieh, and L.M. Chen.

References

1. Gronthos S, Mankani M, Brahim J, et al. Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. Proc Natl Acad Sci U S A 2000;97:13625–30.
2. Gronthos S, Brahim J, Li W, et al. Stem cell properties of human dental pulp stem cells. J Dent Res 2002;81:531–5.
3. Cordeiro MM, Dong Z, Kaneko T, et al. Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J Endod 2008;34:962–9.
4. Gebhardt M, Murray PE, Namerow KN, et al. Cell survival within pulp and periodontal constructs. J Endod 2009;35:63–6.
5. Hargreaves KM, Giesler T, Henry M, et al. Regeneration potential of the young permanent tooth: what does the future hold? J Endod 2008;34(7 Suppl):S51–6.
6. Huang GT, Shagramanova K, Chan SW. Formation of odontoblast-like cells from cultured human dental pulp cells on dentin *in vitro*. J Endod 2006;32:1066–73.
7. Huang GT, Sonoyama W, Liu Y, et al. The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. J Endod 2008;34:645–51.
8. Murray PE, Garcia-Godoy F, Hargreaves KM. Regenerative endodontics: a review of current status and a call for action. J Endod 2007;33:377–90.
9. Wei X, Ling J, Wu L, et al. Expression of mineralization markers in dental pulp cells. J Endod 2007;33:703–8.
10. Sloan AJ, Smith AJ. Stem cells and the dental pulp: potential roles in dentine regeneration and repair. Oral Dis 2007;13:151–7.
11. Huang AH, Chen YK, Lin LM, et al. Isolation and characterization of dental pulp stem cells from a supernumerary tooth. J Oral Pathol Med 2008;37:571–4.
12. Erdmann D, Follmar KE, Debruijn M, et al. A retrospective analysis of facial fracture etiologies. Ann Plast Surg 2008;60:398–403.
13. Bastone EB, Freer TJ, McNamara JR. Epidemiology of dental trauma: a review of the literature. Aust Dent J 2000;45:2–9.
14. Tontonoz P, Hu E, Spiegelman BM. Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid activated transcription factor. Cell 1994;79:1147–56.
15. Cheng PH, Snyder B, Fillos D, et al. Postnatal stem/progenitor cells derived from the dental pulp of adult chimpanzee. BMC Cell Biol 2008;22(9):20.
16. Huang AH, Snyder BR, Cheng PH, et al. Putative dental pulp derived stem/stromal cells promote proliferation and differentiation of endogenous neural cells in the hippocampus of mice. Stem Cells 2008;26:2654–63.
17. Miura M, Gronthos S, Zhao M, et al. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 2003;100:5807–12.
18. Sonoyama W, Liu Y, Yamaza T, et al. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. J Endod 2008;34:166–71.
19. Leco Berrocal MI, Martín Morales JF, Martínez González JM. An observational study of the frequency of supernumerary teeth in a population of 2000 patients. Med Oral Patol Oral Cir Bucal 2007;12:E134–8.
20. Stockwell AJ. Incidence of dental trauma in the Western Australian School Dental Service. Community Dent Oral Epidemiol 1988;16:294–8.
21. Davis GT, Knott SC. Dental trauma in Australia. Aust Dent J 1984;29:217–21.
22. Oulis CJ, Berdouses ED. Dental injuries of permanent teeth treated in private practice in Athens. Endod Dent Traumatol 1996;12:60–5.