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MESENCHYMAL STEM CELL PROPERTIES OF DENTAL PULP CELLS FROM DECIDUOUS TEETH

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Abstract - In the present study we have isolated and identified mesenchymal stem cells (MSCs) from the exfoliated deciduous teeth dental pulp (DP-MSCs), as plastic-adherent, spindle-shaped cells with a high proliferative potential. Immunophenotype analyses revealed that DP-MSCs were positive for mesenchymal cell markers (CD90, CD44, CD105, STRO-1, vimentin and α-SMA), and negative for hematopoietic stem cell markers (CD11b, CD33, CD34, CD45, CD235a). DP-MSCs were also capable of differentiating into adipogenic, chondrogenic, myogenic and osteogenic lineages, fulfilling the functional criterion for their characterization. These results demonstrate that DP-MSCs offer a valuable, readily accessible source to obtain and store adult stem cells for future use.

Key words: Dental pulp, deciduous teeth, mesenchymal stem cells, CFU-F, growth, differentiation

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INTRODUCTION

In recent years there has been a growing emphasis on the use of undifferentiated progenitor cells for tissue engineering owing to their ability to be expanded in culture and to differentiate into multiple cell types. Since the first discovery that adult bone marrow contains rare, yet powerful multipotent progenitor cells (Friedenstein et al.., 1968), often referred to as mesenchymal stem cells (MSCs), multipotent adult progenitor cells, marrow stromal cells, or mesenchymal progenitors (Salem et al., 2010), it is now apparent that many adult tissues harbor cells that have similar characteristics (da Silva Meirelles et al., 2006). These cells exhibit multiple functions and are involved in tissue maintenance and repair, as well as in the regulation of hematopoiesis and immune responses (Chamberlain et al., 2007). Adult MSCs can be defined as undifferentiated cells that can renew themselves and have the ability, once cultured under specific growth conditions, to differentiate into multiple lineages of mesodermal tissues, such as skeletal muscle, bone, tendons, cartilage, and fat, or nonmesenchymal cell-lineages, such as neuron-like cells, hepatocytes and pancreatic-like cells (Prockop et al., 1997). Therefore, MSCs have enormous potential for cell therapies in regenerative medicine.

Bone marrow-derived MSCs (BM-MSCs) remain the most frequently used cells, but alternate sources of MSCs have been extensively sought. Dental tissue has drawn much interest as a potent reservoir of adult MSCs with the potential for cell-mediated therapies and tissue engineering applications. Five different human dental stem/progenitor cells have been isolated and characterized: dental pulp stem cells (DPSCs) (Gronthos et al., 2000), stem cells from human exfoliated deciduous teeth (SHED) (Miura et al., 2003), periodontal ligament stem cells (PDLSCs) (Seo et al., 2004), dental follicle progenitor cells (DF-

PCs) (Morsczeck et al., 2005) and stem cells from apical papilla (SCAP) (Sonoyama et al., 2008). These post-natal populations have MSCs-like qualities, including the capacity for self-renewal and multilineage differentiation potential. As different laboratories use different isolation and *in vitro* culture methods, these variables are often responsible for the phenotype and functional differences of the resulting cell populations. The purpose of the present study was to investigate the potential use of human dental pulp of deciduous teeth as a source of multipotent dental pulp MSCs (DP-MSCs), also referred to as SHED. Therefore, our aim was to characterize isolated and *in vitro* cultured DP-MSCs and to examine their cell differentiation potential.

MATERIALS AND METHODS

Isolation and culturing of DP-MSCs

Deciduous incisors were extracted under local anesthesia according to the approved ethical guidelines set by the Ethical Committee of the School of Dentistry, University of Belgrade, with informed consent obtained from the parents of patients. The pulp tissue was separated from the tooth crown with the appropriate dental instruments (excavators). Isolated pulp tissues were kept in Dulbecco's modified Eagle's medium (DMEM, Sigma St. Louis, MO, USA) supplemented with 10% Fetal Bovine Serum (FBS, PAA Laboratories, Linz, Austria) and delivered to the laboratory for the isolation of DP-MSCs. After centrifugation for 10 min at 1800 rpm and supernatant removal, extracted pulp tissues were subsequently digested in a solution of 3 mg/ml collagenase type I (Sigma–Aldrich, Saint Louis, MO, USA) in Phosphate-Buffered Saline (PBS, PAA Laboratories, Linz, Austria), supplemented with 20% FBS for 45 min at 37°C. Afterwards, PBS containing 2% FBS was added to cell suspensions that were pelleted by centrifugation for 10 min at 1,800 rpm and enumerated for viable cells by the trypan blue dye exclusion test.

As described previously (Gronthos et al., 2000), the DP-MSCs were isolated based on their ability to adhere to culture plates. Namely, DP-MSCs obtained from one tooth were seeded into 25 cm^2 plastic tissue culture flasks (Sarstedt, Numbrecht, Germany) (0.1 - 1 x 10^4 cells/cm^2) and cultured in a growth medium (GM) containing DMEM, 20% FCS, 200 μM L-ascorbic acid-2-phosphate (Sigma, St Louis, MO, USA), 100 units⁄ml penicillin/streptomycin (PAA Laboratories, Linz, Austria) at 37°C in a humidified atmosphere containing 5% CO_2. After 3 days, non-adherent cells were removed and fresh medium was added to allow further growth. Fresh medium was replaced every 2-3 days and the cells were left to grow to subconfluency (80-90% of surface occupancy). For passaging, the adherent cells were washed with Ca^{2+}/Mg^{2+}-free PBS and detached by incubating with 0.25 % trypsin-EDTA solution (PAA Laboratories, Linz, Austria) for 5-10 min at 37°C. Subsequently, a growth medium containing FBS was added and the detached cells centrifuged at 1,800 rpm for 10 min. Pelleted cells were then resuspended in GM, counted for viable cells using the trypan blue exclusion test, and plated for the next passage in 25 cm^2 flasks at a concentration of 1×10^4 cells/cm^2.

CFU-F (Colony Forming Unit-Fibroblast) assay

CFU-F assays were performed by plating third to sixth passage DP-MSCs in a 6-well plate at 10, 50 and 100 cells/well in GM with two replicas. After 14 days in culture at 37°C in a humidified atmosphere containing 5% CO_2, the cells were washed twice with PBS, fixed with ice-cold methanol for 5 min at room temperature, and stained with 0.3% crystal violet for 15 min. The cells were washed with distilled water and the number of colonies was counted. Colonies greater than 2 mm in diameter were enumerated.

Cell growth assay

Short-term cell growth assays were performed by seeding DP-MSCs (passage [P] 2 to 5) in a 6-well plate at 4 x 10^4 cells/well in duplicate, and incubating them in GM for seven days at 37°C in a humidified atmosphere containing 5% CO_2. At days 2, 4, and 7, the cells were harvested by trypsin-EDTA treatment and washed in GM through centrifugation (1,500 rpm for 10 min). The pelleted cells were then count-

ed with a hemocytometer and their viability determined by the trypan blue dye exclusion test. These assays were repeated at least 3 times for each donor.

To determine the long-term population doubling times at various cell densities, we seeded DP-MSCs (P2 to P5) in a 6-well plate at 1 x 10^4, 5 x 10^4 or 1 x 10^5 cells/well, and incubated them at 37°C in a humidified atmosphere containing 5% CO_2. At confluence they were detached by trypsin-EDTA treatment, counted and reseeded at the initial cell density. This procedure was repeated at every passage for 24 days. The population doubling times were calculated according to the formula PDT=(T-T0) lg2/(lgNt-lgN0), where PDT is the population doubling time, T0 is the starting time of cell culture and T is the ending time of cell culture, while N0 and Nt separately are the cell numbers at the start and the end of each culture, respectively.

Flow cytometry

To confirm the mesenchymal stromal phenotype of isolated and *in vitro* expanded dental pulp cells, third to sixth passage DP-MSCs were subjected to flow cytometry analysis for expression of positive (CD44H, CD90, CD105, STRO-1) and negative (CD33, CD45, CD34, CD11b, CD235a) MSC markers. The cells, harvested by trypsin-EDTA for 10 min, were washed in cold PBS supplemented with 0.5% Bovine Serum Albumine (BSA; Sigma-Aldrich, Saint Louis, MO, USA), and aliquots of 2 × 10^5 cells were labeled (30 min in the dark at 4°C) with monoclonal antibodies specific for human markers associated with mesenchymal and hematopoietic lineages. Namely, immunophenotyping of DP-MSCs was performed with mouse anti-human antibodies against the following antigens: CD34 (Hematopoietic Progenitor Cell Antigen; PE conjugated) (Dako Cytomation, Glostrup, Denmark), CD11b (Mac-1 α; FITC conjugated), CD45 (leukocyte common antigen/cell marker of hematopoietic origin; FITC conjugated), CD105 (endoglin; R-PE conjugated), (Biosource Invitrogen, Camarillo, CA), CD33 (Sialic Acid-Binding Immunoglobulin-Like Lectin 3; SIGLEC3; a surface marker for very early bone marrow-derived hematopoietic stem cells; Fluorescein conjugated), CD235a (glycophorin A; PE conjugated), CD90 (Thy-1/Thy-1.1; PE conjugated), CD44H (PE conjugated), STRO-1 (unconjugated) (R & D Systems, Minneapolis, MN, USA). The binding of unconjugated STRO-1 antibody was visualized by adding the secondary goat anti-mouse IgM antibody conjugated to fluorescein (R & D Systems, Minneapolis, MN, USA). To determine the level of nonspecific binding, fluorochrome-conjugated isotype control antibodies were used. Flow cytometry was performed using a CyFlow CL (Partec, Münster, Germany).

Immunofluorescence

DP-MSCs were seeded over rounded coverslips in GM and cultured for 24 h. After fixation with 4% formaldehyde in PBS, cell monolayers were treated with PBS-0.1% Triton X for 2 min to permeabilize the cell membrane, and then incubated for 1 h at room temperature with mouse anti-vimentin and mouse anti-α-SMA (alpha-Smooth Muscle Actin) antibody (Santa Cruz Biotechnology, CA, USA), followed by 1 h incubation in the dark with an anti-mouse-FITC secondary antibody (Santa Cruz Biotechnology, CA, USA) and 1 μg/ml DAPI (Sigma-Aldrich, Saint Louis, MO, USA). The samples were observed and photographed using an epifluorescence microscope.

In vitro differentiation of DP-MSCs

To determine the differentiation potential of DP-MSCs, third to fifth passage cells were seeded in a 24-well plate at 4,000 cells/cm^2 and cultured in the GM until the monolayer cultures achieved subconfluence. At this point, the basal medium was replaced with a specific differentiation medium in order to induce differentiation into different mesenchymal lineages. As control, the growth medium only was used.

For adipogenic differentiation, subconfluent DP-MSCs monolayer cultures were incubated in an adipogenic medium consisting of DMEM supplemented with 100 μg/ml isobutylmethylxanthine (IBMX; Sigma-Aldrich, Saint Louis, MO, USA), 1 μM dexamethasone, 10 μg/ml insulin (Actrapid, Novonor-

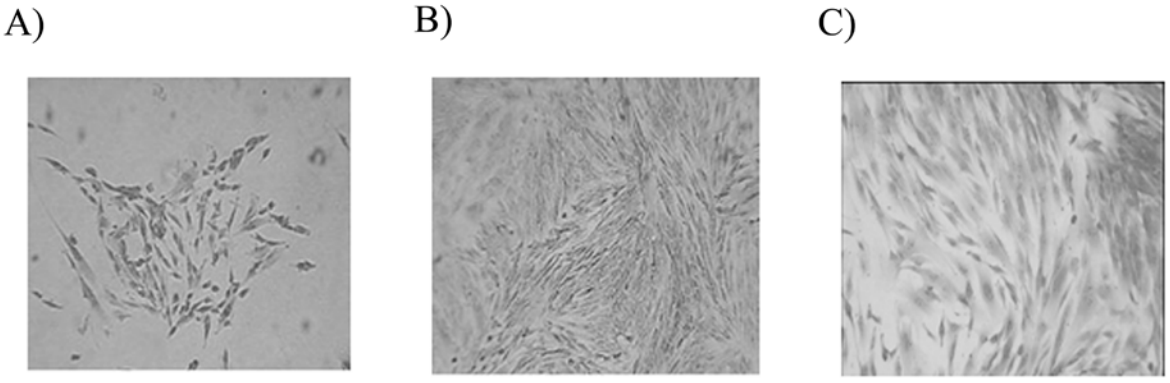

Fig. 1 Morphologic characteristics of DP-MSCs. (A) DP-MSCs starting to form small colonies; (B) DP-MSCs at confluency, ready for first passage; (C) Typical fibroblast-like, spindle-shaped morphology of the cells.

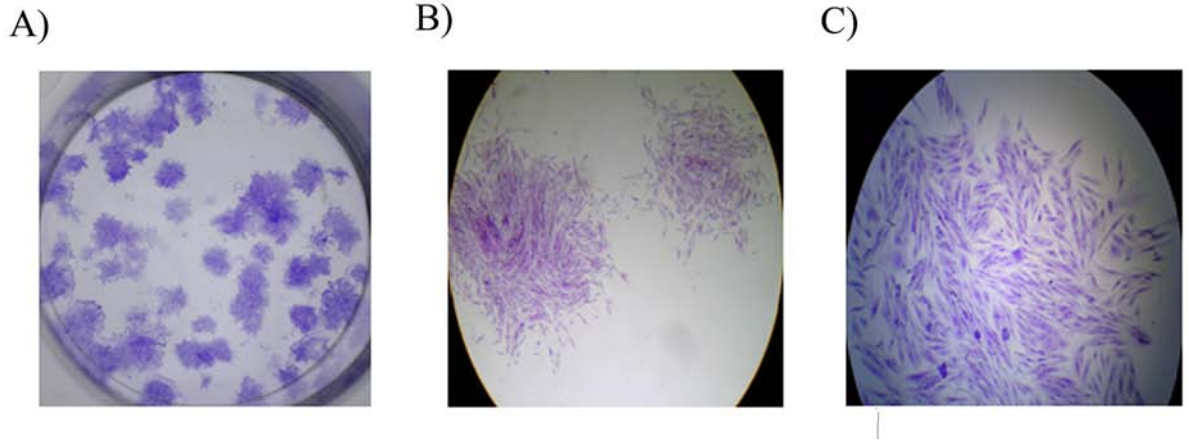

Fig. 2 CFU-F assay. DP-MSCs were cultivated in GM for 14 days and stained with crystal violet. (A) Multiple colonies after 14 days in culture; (B) Clonogenic colonies originates from a single cells; (C) Single colony of DP-MSCs with typical fibroblast-like morphology of the cells.

disc, Bagsvaerd, Denmark), 100 U/ml penicillin/streptomycin, 1% HEPES and 10% FBS, for 4 weeks. The medium was replaced three times per week. The presence of intracellular lipid droplets, which indicates adipogenic differentiation, was confirmed by Oil Red O (Merck, Darmstadt, Germany) staining.

To induce chondrogenic differentiation, the basic medium from the subconfluent DP-MSCs monolayer cultures was replaced with a chondrogenic medium containing DMEM supplemented with 5 ng/ml transforming growth factor-β1 (TGF-β1; R & D Systems, Minneapolis, MN, USA), 200 μM ascorbic acid-2-phosphate, 10 nM dexamethasone, 100 U/ml penicillin/streptomycin, 1% HEPES and 1% FBS. The cultures were incubated for another 21 days with the medium being changed three times a week. At the end of the incubation, chondrogenic differentiation of the cells was assessed via staining with Safranin O (Merck, Darmstadt, Germany).

To induce myogenic differentiation, DP-MSCs were cultured in a myogenic medium DMEM supplemented with 5% horse serum (PAA Laboratories, Linz, Austria), 50 μM hydrocortisone (Galenika, Beograd, Serbia), 0.1 μM dexamethasone and 2% FBS. The cells were allowed to grow in this medium for 16 days with the medium changed every 2-3 days. In order to assess myogenic differentiation, the cells were washed with PBS, fixed with ice-cold methanol

for 5 min at room temperature, and stained with 0.3% crystal violet for 15 min. The cells were washed with distilled water and examined for the presence of myotubes containing three or more aligned nuclei using a light microscope.

For osteogenic differentiation, DMEM was supplemented with 10 nM dexamethasone (Applichem, Darmstadt, Germany), 200 μM ascorbic acid-2-phosphate, 10 mM β-glycerophosphate (Sigma–Aldrich, Saint Louis, MO, USA), 100 U/ml penicillin/streptomycin, 1% HEPES (PAA Laboratories, Linz, Austria) and 10% FBS. The cells were incubated in a humidified atmosphere at 37°C and 5% CO_2 and the medium was replaced three times a week. On days 6 and 7, osteogenic differentiation was analyzed via assessment of alkaline phosphatase activity by staining with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium, BICP/NBT (Sigma-Aldrich, Saint Louis, MO, USA). The cells were then photographed using a light microscope.

RESULTS

Isolation and culturing of DP-MSCs

To isolate DP-MSCs, single cell suspensions were derived from the remnant pulp of exfoliated deciduous teeth after collagenase treatment, and placed in a liquid culture at low cell density (0.1 - 1 x 10^4 cells/cm^2), depending on the number of dental pulp cells obtained from the tissue sample. Cell growth, morphology and shape were followed and examined under an inverted microscope. One-to-two days after plating, the cells started to aggregate and adhere to a plastic dish. The majority of the cells displayed a

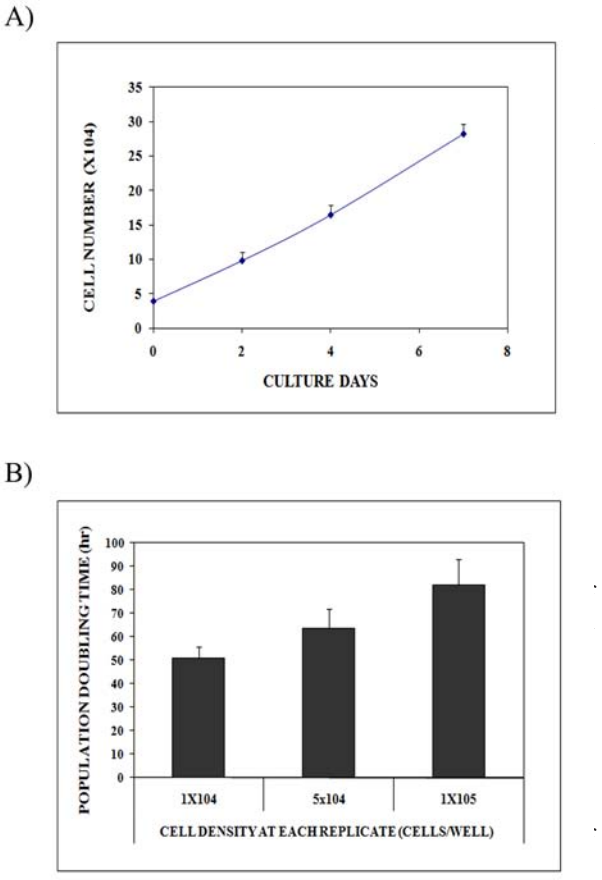

Fig. 3 A) Short-term proliferation of DP-MSCs. DP-MSCs were seeded in GM at 4x10^4 cells/well in duplicate and after 2, 4, and 7 days cells were detached by trypsinization and counted; B) Proliferation of DP-MSCs in long-term cultures at various cell densities. DP-MSCs were grown in GM at 1x10^4, 5x10^4 or 1x10^5 cells/well, passaged at 90% confluency and counted at each passage during 24 days. PDTs were calculated according to the formula described in Material and Methods. The results in A) and B) are expressed as the mean ± SE of triplicate determinations. Significant difference for PDTs from the cultures seeded at 1x10^4 cells/well by t-test: *$p<0.05$.

Table 1. The incidence of colony-forming cells from dental pulp tissue at various plating densities.

	Cells seeded		
	10	50	100
number of CFU-F (mean ± SE)	8.25 ± 3.22	29.5 ± 4.29	40.25 ± 7,98

DP-MSCs were cultivated at low density (10, 50 or 100 cells/well) in growth medium for 14 days and stained for CFU-F with crystal violet. The results are presented as mean ± SE for three experiments each performed in duplicate.

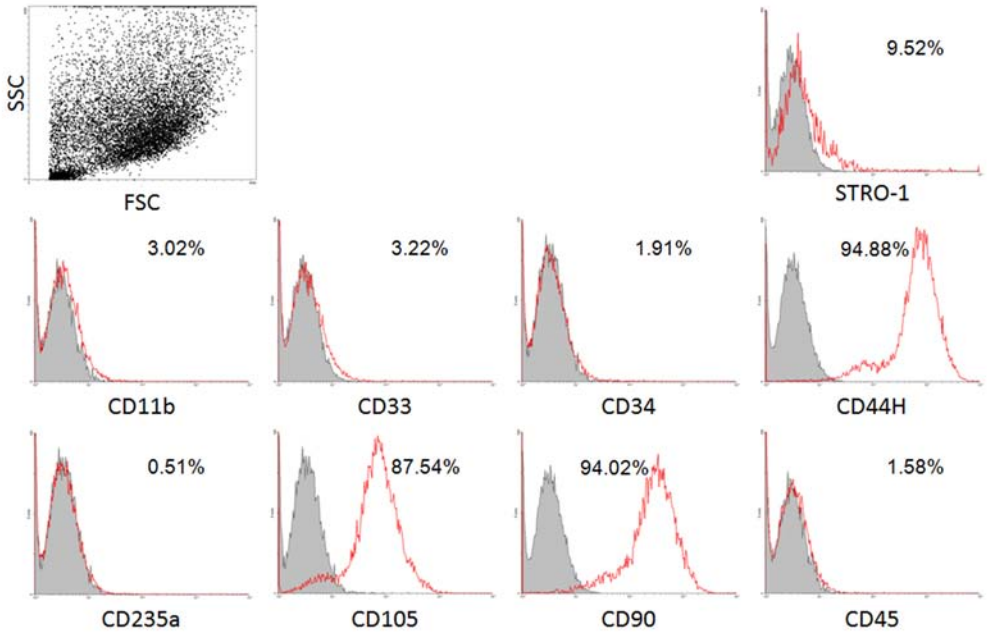

Fig. 4 Flow cytometry analysis of the expression of cell surface markers related to mesenchymal (CD44, CD90, CD105, STRO-1) or hematopoietic stem cells (CD11, CD33, CD45, and CD235a). The gray filled histogram represents the background staining obtained with an isotype-matched control antibody. Representative histogram plots for the detection of cell surface antigens.

ranged from an average of 8.25 CFU-F per 10 cells plated up to 40.25 CFU-F per 10^2 cells plated (Table 1), demonstrating that more than 80% of DP-MSCs were capable of forming colonies when seeded at low cell density.

Proliferation assay

A short-term proliferation assay demonstrated a high proliferation rate of DP-MSCs, as the cell number increased significantly on day two, while at day seven the loading of 4 x 10^4 cells resulted in a six-fold increase of the proliferation rate (Fig. 3A).

The analysis of DP-MSCs proliferation in long-term cultures demonstrated an increase in population doubling times (PDT) dependent on the initial cell plating density (Fig. 3B), indicating that the growth kinetics of DP-MSCs is dependent on the cell numbers plated. Estimation of PDT over a period of 24 days revealed a significantly higher PDT (of approximately 60%) of the DP-MSCs seeded at 1x10^5/well in comparison to DP-MSCs seeded at 1x10^4/well. Indeed, DP-MSCs plated at 1x10^5/well displayed a PDT of 80 h, while DP-MSCs plated at 1x10^4/well grew much faster with a PDT of 50 h.

Immunophenotype analyses

For further characterization of the DP-MSCs, surface protein expression was examined by flow cytometry (Fig. 4) and immunofluorescence staining (Fig. 5). The analyses revealed a positive expression of the markers related to mesenchymal stem cells, with constantly strong positive results (more than 90% positive cells) of the CD44, CD90 and CD105 markers. In addition, the early mesenchymal cell marker STRO-1 was also positive, but in a lower percent of cells. Cell-surface markers that were negative (less than 2% positive cells) were related to hematopoietic stem cells and included CD11b, CD33, CD34, CD45, and CD235a (Fig. 4).

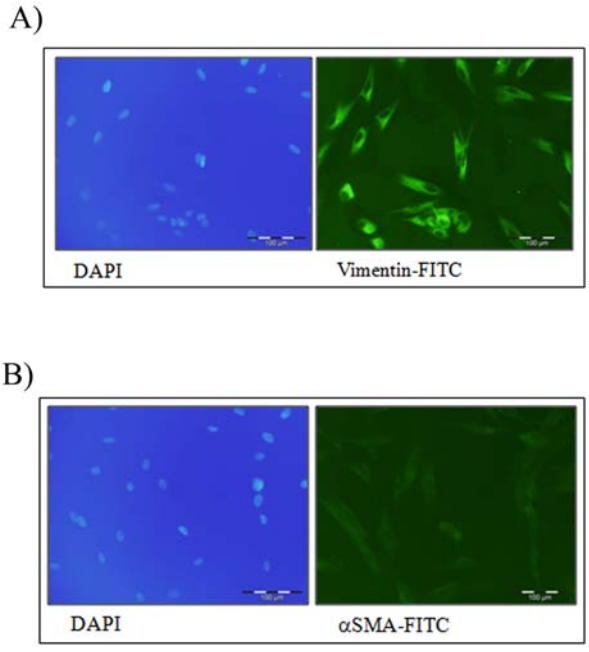

Fig. 5 Immunofluorescence staining for vimentin (A) and α-SMA (B). Positive cytoplasmic staining of DP-MSCs for intracellular MSCs markers by indirect immunofluorescence with mouse anti-vimentin and mouse anti-α-SMA antibody and anti-mouse FITC secondary antibody (green); nuclei were counterstained with DAPI (blue). Cells were examined using an immunofluorescence microscope (400x magnification).

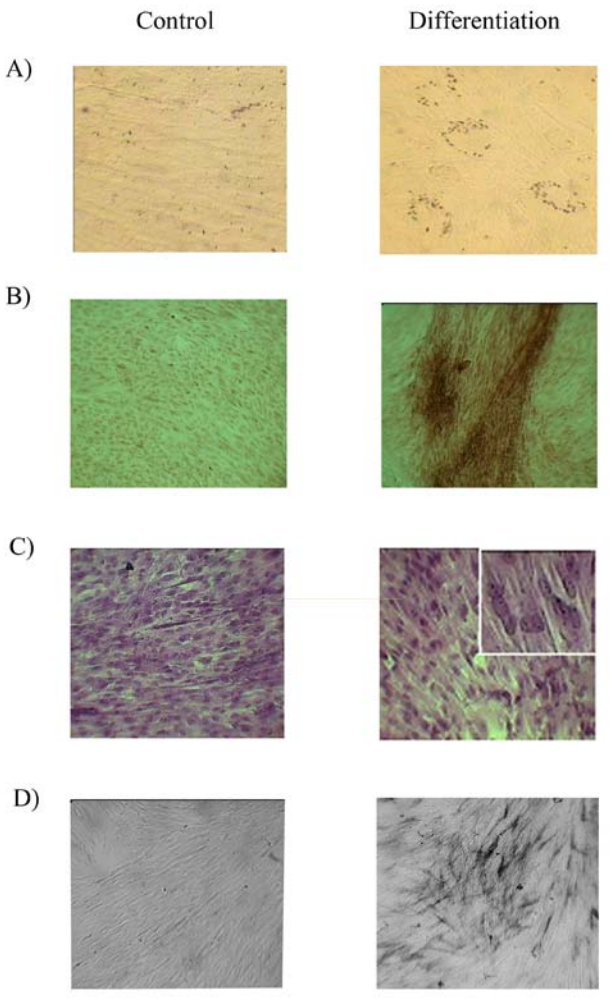

Fig. 6 Differentiation ability of DP-MSCs**.** (A) Adipogenic differentiation of DP-MSCs was confirmed by Oil Red O staining of intracytoplasmatic lipid droplets; (B) Chondrogenic differentiation of DP-MSCs with positive staining of proteoglycans by Safranin O; (C) Myogenic differentiation of DP-MSCs characterized by the formation of myotubes stained with crystal violet. Insert at the top shows higher magnification; (D) Osteogenic differentiation DP-MSCs was proved by positive staining for ALP.

In control culture conditions Oil Red O staining was all negative (Fig. 6A). To assess the chondrogenic differentiation ability of DP-MSCs, secreted cartilage-specific proteoglycans were stained with Safranin O. In DP-MSCs grown under chondrogenic conditions the presence of chondrogenic proteoglycans was observed after 21 days of culture and was confirmed by positive Safranin O staining, whereas, as expected, control cultures grown in the regular medium were negative for Safranin O staining (Fig. 6B). The myogenic differentiation capacity of DP-MSCs was confirmed by the formation of multinucleated myotubes after 16 days of incubation in a myogenic differentiation medium. As shown in Fig. 6C, the myotubes formed were stained with crystal violet, while these were not observed in the control cultures incubated in the regular growth medium. As a marker for osteoblast differentiation capacity, alkaline phosphatase (ALP) levels were measured. Osteogenic differentiation was confirmed by the detection of an osteogenic phenotype characterized by an increased expression of ALP. In fact, ALP activity in the cultured DP-MSCs following a 7-day incubation in an osteogenic differentiation medium was remarkably increased compared with cells exposed to the regular growth medium (Fig. 6D).

DISCUSSION

In the present study we provide evidence that dental pulp from exfoliated deciduous teeth contains clonogenic, highly proliferative cells capable of multilineage differentiation. As no definite method has been introduced to identify mesenchymal stem cells, we have followed the criteria for defining multipotent mesenchymal stromal cells recommended by the International Society for Cellular Therapy (ISCT), i.e. the plastic-adherent behavior of the cells when maintained in standard culture conditions, cellular morphology assessments, immunophenotype analyses, as well as the differentiation capacity of cells as the most accurate technique to confirm their nature of stem cells. The isolated and expanded DP-MSCs showed characteristics similar to those typically described for MSCs isolated from various sources, mostly from bone marrow. Their property to adhere to plastic has been used to isolate MSC-like cells, while the microscopic examination of the expanded cells confirmed their spindle elongated shaping, with no observable changes in their morphology and growth patterns in subsequent passages. The capacity to generate clones and the resulting high cloning efficiency (up to 80%) was another important characteristic of the expanded DP-MSCs, and was in a good agreement with previous observations made on dental pulp stem cells from both mesiodens and deciduous teeth (Huang et al., 2008). In addition, the obtained cells also showed a high proliferation rate, attributed to the developmental state of the investigated tissue. The extensive proliferation capacity was maintained after multiple subculturing, which is a very important feature for their potential application in cell mediated therapies.

As an index of stem cell identity, immunophenotype analyses was also proposed by ISCT, and both positive and negative surface antigen expressions of the cells were considered. Flow cytometric analysis showed that DP-MSCs were strongly positive (ca. 90% and above) to cell markers of mesenchymal cells, such as CD90 (also known as Thy1), CD44 (an integral membrane protein interacting with components of extracellular matrix) and CD105 (known as endoglin). While the early mesenchymal cell marker STRO-1 was positive in a smaller percentage of cells, it was in agreement with previously reported data (Miura et al., 2003). Moreover, as demonstrated by immunofluorescent staining, DP-MSCs expressed α-SMA and vimentin, intracellular markers that are additional indicators of mesodermally derived tissues. Furthermore, to assure that DP-MSCs were not contaminated with other cells, we used CD11b, CD33, CD34, CD45, CD235a markers to exclude the presence of hematopoietic cells that can be found in MSC cultures. CD11b is prominently expressed on monocytes and macrophages, the most likely hematopoietic cells to be found in an MSC culture; CD45 is a pan-leukocyte marker; CD34 marks primitive hematopoietic progenitors; CD33 is expressed on cells of myeloid lineage, while CD235a is present in human red blood cells and erythroid precursors. As expected, DP-MSCs were negative for all these markers.

Positivity or negativity of markers in flow cytometric analysis cannot be used as the ultimate confirmation of the stem cell nature of the present cells, albeit it can increase the probability of cells to be stem in nature. Therefore, the biological property that most uniquely identifies MSC, i.e. their capacity for multi-lineage mesenchymal differentiation, was used as a final confirmation of their stem cell nature. MSCs are traditionally considered to be capable of differentiating into cell types of their own original lineage only, i.e. mesenchymal derivatives. Bone marrow-derived MSCs were previously shown to differentiate into osteogenic, chondrogenic, adipogenic, myelosupportive stroma, myogenic, and neurogenic lineages, but the first four cell types mentioned are considered to be the essential lineages for defining multipotent MSCs (Tuli et al., 2003). The myogenic potential of MSCs was emphasized in reports demonstrating the delivery of bone marrow MSCs into muscle tissues, especially the myocardium (Ferrari et al., 1998; Gojo et al., 2003). We were also able to induce osteogenic, chondrogenic, myogenic and adipogenic differentiation of the DP-MSCs under specific differentiation media, thus identifying the genuine MSCs and distinguish-

ing them from precursor cells, such as preosteoblast, preadipocyte or prechondrocytic cells which each only give rise to one cell type (Pittenger et al., 1999).

Stem cells from human exfoliated deciduous teeth, often referred to as SHED, were first identified in 2003 as a population of heterogeneous cells (Miura et al., 2003), with many differences from MSCs originating from the pulp of permanent teeth (Miura et al., 2003; Nakamura et al., 2009), but consistent with the differences existing between deciduous and permanent teeth in regard to their developmental processes, tissue structure and function. The DP-MSCs characterized in our study are comparable to the other reported stem cells found in the primary teeth of children-SHED (Miura et al., 2003, Arora et al., 2009). SHED are potentially superior to other types of adult/postnatal stem cells in terms of their high proliferation capability and abundant cell supply (Wang et al., 2010). Since these cells often multiply rapidly and grow much faster than stem cells isolated from other adult tissues, it was even suggested that they are less mature, and might have the potential to develop into a variety of tissue types (Miura et al., 2003). All these characteristics open up new opportunities for even more therapeutical applications of stem cells, since stem cells from an easily accessible and available source are indispensable for tissue engineering and clinical applications. With the establishment of cellular banks, the multiple advantages of banking stem cells from exfoliated deciduous teeth has come into the foreground, as these are adult stem cells that are not subject to the ethical concerns of embryonic stem cells, they provide a matching donor (autologous transplant) for life, can be saved before damage occurs, collection is simple and painless with minimal invasion, for both children and parents, and above all they are complementary to the mesenchymal stem cells derived from other adult and post-natal tissues. The ordered replacement of twenty deciduous teeth may take more than seven years in humans, making them an available source of MSCs for a long period. Until we can overcome the obstacle of immune rejection, these cells may be a valuable resource for personalized medicine that uses a patient's own stem cells for biologically compatible therapies and individually tailored treatments (Arora et al., 2009).

In conclusion, in the present study we report the isolation and characterization of stem cell cultures from human dental pulp of deciduous teeth. Our findings clearly demonstrates that isolated DP-MSCs express mesenchymal cell markers and can differentiate into osteogenic, adipogenic, myogenic and chondrogenic cells depending on the culture conditions. Although further investigations are needed to confirm their functional properties *in vivo*, it is clear that the DP-MSCs obtained are a promising potential source for tissue engineering not only in the craniofacial region.

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