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Mesenchymal and embryonic characteristics of stem cells obtained from mouse dental pulp

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ABSTRACT

Objective: Several studies have demonstrated that human dental pulp is a source of mesenchymal stem cells. To better understand the biological properties of these cells we isolated and characterized stem cells from the dental pulp of EGFP transgenic mice.

Methods: The pulp tissue was gently separated from the roots of teeth extracted from C57BL/6 mice, and cultured under appropriate conditions. Flow cytometry, RT-PCR, light microscopy (staining for alkaline phosphatase) and immunofluorescence were used to investigate the expression of stem cell markers. The presence of chromosomal abnormalities was evaluated by G banding.

Results: The mouse dental pulp stem cells (mDPSC) were highly proliferative, plastic-adherent, and exhibited a polymorphic morphology predominantly with stellate or fusiform shapes. The presence of cell clusters was observed in cultures of mDPSC. Some cells were positive for alkaline phosphatase. The karyotype was normal until the 5th passage. The *Pou5f1/Oct-4* and *ZFP42/Rex-1*, but not *Nanog* transcripts were detected in mDPSC. Flow cytometry and fluorescence analyses revealed the presence of a heterogeneous population positive for embryonic and mesenchymal cell markers. Adipogenic, chondrogenic and osteogenic differentiation was achieved after two weeks of cell culture under chemically defined *in vitro* conditions. In addition, some elongated cells spontaneously acquired a contraction capacity.

Conclusions: Our results reinforce that the dental pulp is an important source of adult stem cells and encourage studies on therapeutic potential of mDPSC in experimental disease models.

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1. Introduction

During the embryonic developmental stage, epithelial–mesenchymal interactions determine the formation of all the dental components, including the pulp.[1] The pulp is divided into four layers: the external layer is constituted by odontoblasts which produce the dentine. The dentine keeps and protects the inner dental pulp chamber, comprised by the second layer, a zone poor in cells and rich in extracellular matrix, and the third layer containing compact connective tissue. The last layer is infiltrated by a vascular area and a nervous plexus.[2,3] The presence of undifferentiated cells around the vessels, responsible for the new dentine formation after dental injuries such as cavities or mechanical trauma, has highlighted the dental pulp as a source of mesenchymal stem cells.[1,2] Of particular interest is the fact that rodent incisors grow continually, unlike rodent molars and human teeth. The apical part is responsible for the enamel matrix production. This area contains epithelial stem cells that originate the ameloblasts, stratum intermedium, stellate reticulum and outer dental epithelium layers.[4]

The first identification and isolation of precursors of functional odontoblasts known as human dental pulp stem cells (DPSC) was reported in by Gronthos et al.[5] These cells were characterized by their highly proliferative capacity, the typical fibroblast-like morphology, multipotent differentiation, the expression of mesenchymal stem cells markers *in vitro,* as well as by dentine regeneration induction in vivo.[6] Several other populations of human dental stem cells have been characterized, such as stem cells obtained from deciduous teeth,[6,7] apical papilla,[8] and periodontal ligament stem cells.[9,10] Cell populations obtained from rat dental pulp contain STRO-1 positive cells with multilineage potential of differentiation *in vitro*.[11] A recent study demonstrated that erupted murine molars contain a population of multipotent cells with osteogenic, adipogenic, and chondrogenic differentiation abilities.[12] Other reports have described the gene expression pattern associated with the regulation of the tooth germ morphogenesis in the mouse incisor.[13,14]

A study performed by Balic and Mina[34] provided evidence that dental pulp tissue obtained from unerupted and erupted murine incisors contains a progenitor, but not a multipotent mesenchymal stem cell population. In this work, we performed a detailed description of stem cells from the incisor dental pulp of continuous growth obtained from EGFP transgenic C57BL/6 mice with mesenchymal and embryonic characteristics. GFP expression constitutes an important tool for the study of stem cells *in vitro* and in vivo. The results confirm that mDPSC have properties that effectively define them as stem cells.

2. Materials and methods

2.1. Mice

Specific-pathogen-free, 8-week-old male enhanced green fluorescent protein (EGFP) transgenic C57BL/6 mice were maintained at the animal facilities at the Gonçalo Moniz Research Center-FIOCRUZ, Salvador, Bahia, Brazil, and provided with rodent diet and water *ad libitum*. The present study was approved by the Institution's Animal Ethics Committee.

2.2. Isolation of mDPSC and cell culture

The incisors teeth were dissected carefully from the mandibles of male EGFP transgenic C57BL/6 mice after removal of the heads under deep anaesthesia in the CO_2 chamber. Special care was taken to avoid contamination by adjacent tissues. Whole dental pulp tissue was gently collected with the aid of a stereotactic microscope (Olympus, Tokyo, Japan), washed three times with sterile saline, and transferred into 24-well plates (Nunc A/S, Roskilde, Denmark). The growth medium consisted of Dulbecco's Modified Eagle Medium – DMEM medium supplemented with 10% foetal bovine serum (FBS; Cultilab, Campinas, SP, Brazil), 23.8 mM sodium bicarbonate (Sigma, St. Louis, MO, USA), 10 mM Hepes (Santa Cruz Biotechnology, Santa Cruz, CA, USA), 1 mM sodium pyruvate (Sigma), 2 mM L-glutamine (Sigma), 0.05 mM 2-mercaptoethanol (Sigma), 50 μg/ml gentamycin (Sigma), and incubated at 37 °C with 5% CO_2. Pieces of tissue explant were used to isolate mDPSC. Culture medium was replaced every 3–4 days. After confluence (usually after 15–20 days), the adherent cells were released with 0.25% trypsin solution (Invitrogen/Molecular Probes, Eugene, OR, USA) and re-plated (passages) or used in experimental assays, as described below. For cryopreservation, cells were centrifuged and the pellet was resuspended in DMEM medium supplemented with 10% FBS and 10% dimethylsulfoxide (Sigma). Aliquots (5×10^6 cells/ml) were transferred to cryogenic tubes and cooled slowly until −80 °C and, after 24 h, the cryotubes were transferred to a liquid nitrogen container for long-term storage. Cells of the same isolate in different passages were used in the experiments.

2.3. Karyotype analysis

Cytogenetic analysis of mDPSC metaphases was taken in the 1st and 5th passages, after expansion in the growth medium supplemented with 10% FBS (Cultilab). Cells undergoing active cell division were blocked at metaphase by 0.3 μg/mL colcemid (Cultilab), detached from the growth surface by 0.25% trypsin solution (Invitrogen), and subsequently swollen by exposure to 0.075 M KCl hypotonic solution (Merck). The cells were then fixed in methanol/acetic acid solution (3:1) for slide preparation. Chromosomal analysis of metaphases cells was performed by G banding.[15] The images were captured on BX61 microscope (Olympus, Tokyo, Japan), Spectral Imaging Band View software (Applied, Vista, CA, USA). For each passage, in average fifteen to twenty cells were analysed.

2.4. Flow cytometric analysis

For detection of surface antigen, adherent cells were detached with 0.25% trypsin solution (Invitrogen), washed with saline and incubated at 4 °C for 30 min with following antibodies diluted 1:100: biotin anti-mouse CD31 (BD Biosciences Pharmingen, San Diego, CA, USA), biotin anti-human stromal stem cells – STRO-1 (R&D Systems, Minneapolis, MN, USA), PE anti-mouse CD34 (Invitrogen), PE anti-mouse/human oct-4 (BD Pharmingen), PE anti-mouse CD73 (BD Pharmingen), PE anti-mouse CD90 (Invitrogen), PE anti-mouse CD11b (BD Pharmingen), PE anti-mouse CD44 (BD Pharmingen), PE anti-mouse CD117 (Invitrogen), APC anti-mouse CD45 (Invitrogen), PE-

Cy5.5 anti-mouse stem cell antigen – Sca-1 (Invitrogen) or 0.5 μg/mL propidium iodide (BD Pharmingen). Excess antibody was removed by washing. Streptavidin PE-Cy5.5 diluted 1:100 (BD Pharmingen) was used after biotin antibody. Cells were fixed with 1% formaldehyde. Quantitative evaluation of the exponential cell expansion was estimated by Carboxyfluorescein succinimidyl ester – CFSE assays (Invitrogen/Molecular Probes). CFSE staining was performed according to methodology previously described.[16] The acquisition and analysis were done using a FACScalibur cytometer (Becton Dickinson, San Diego, CA, USA) with the CellQuest software. At least 50,000 events were collected.

2.5. Cytochemistry

Alkaline phosphatase expression was evaluated in monolayers of cells in the third passage cultivated in 24 well plates. USP-1, a mouse embryonic stem cell line[17] was used as a positive control. Cultures were washed with PBS, fixed with 4% paraformaldehyde (Sigma) in PBS, washed with rinse buffer, and stained with a mix fast red violet (FRV) with naphthol phosphate solution and water as described in the protocol of the embryonic stem cell characterization kit (Millipore Corporation, Billerica, MA). Positive alkaline phosphatase expression was identified by red cell colonies visualized using an inverted optic microscope (Olympus).

2.6. Immunofluorescence

For immunofluorescence analysis, 13-mm diameter glass coverslips (Knittel, Braunschweig, Germany) were placed in a 24-well plate and mDPSC (5×10^6) were added in each well. Cells were washed in PBS 1×, fixed with 4% paraformaldehyde and permeabilized with 0.1% triton X-100 for 10 min. After blocking with PBS containing 5% BSA (Sigma), the cells were incubated with primary antibodies diluted 1:100. The embryonic stem cell characterization kit (Chemicon, Temecula, CA, USA) was used for detection of the following primary antibodies: SSEA-1 (stage-specific embryonic antigen-1; IgM monoclononal antibody), SSEA-4 (IgG monoclononal antibody), TRA-1-60 (keratin sulfate-associated antigens; IgM monoclononal antibody). After washing, appropriate secondary antibodies goat anti-mouse IgG or IgM Alexa Fluor 568 (Invitrogen/Molecular Probes) diluted 1:200 were added in the well. Incubations with both primary and secondary antibodies were performed for 1 h at room temperature. The slides were cover slipped using Vectashield mounting medium with 4′,6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA, USA). Images were analysed in confocal microscope Fluowiel 1000 (Olympus) using appropriate filters.

2.7. RT-PCR

Total RNA was isolated using Rneasy® Mini Kit – Qiagen, USA. Total RNA was eluted from the mini columns with 30 μl of RNase-free water. RNA concentrations and purity were measured with a spectrophotometer (NanoDrop Technologies, EUA). To remove any residual DNA, the purified RNA was treated with DNase I, Amp Grade (Invitrogen). The cDNA was synthetized with oligo dT (Invitrogen), following DTT 0.1 M (Invitrogen) and enzyme Super Script II (Invitrogen) incubated for 2 h at 42 °C. The enzyme was inactivated by heating at 70 °C for 15 min. The following primers were used for amplification by RT-PCR: *ZFP42/Rex1*, transcription factor, forward primer 5′-GGTGAGTTTTCYSAACCCA-3′ and reverse primer 5′-YGAWACGGCTTCTCTCC-3′ (annealing temperature 60 °C); *Nanog*, transcription factor, forward primer 5′-GTCTKCTRCTGAGATGC-3′ and reverse primer 5′-ASTKGTTTTTCTGCCACC-3′ (55 °C). Reverse transcriptase polymerase chain reaction (RT-PCR) was carried out as described previously.[7] RT-PCR products were separated by electrophoreses in 2% agarose gel, and visualized under UV-light after ethidium bromide staining.

2.8. Differentiation assays

The potential of differentiation into osteogenic, chondrogenic and adipogenic lineages was examined. To promote osteogenesis, the cells were incubated with DMEM supplemented with 10 mM β-glycerol phosphate (Sigma), 0.05 mM ascorbate-2-phosphate (Sigma) and 100 nM dexamethasone (Sigma). The culture medium was changed twice a week for up to two weeks. To calcium detection, the cells were fixed with methanol for 10 min at room temperature and stained with alizarin red (Sigma) with pH 4.0 for 5 min at room temperature to evaluate the presence of calcium. For adipogenesis, the cultured cells were incubated in adipogenic medium DMEM supplemented with 60 mM indomethacin, 0.5 mM dexamethasone, and 0.5 mM isobutylmethylxanthine (Sigma). The culture medium was changed two times per week for up to three weeks. The cells then were fixed in methanol for 45 min and stained with Oil Red (Sigma). Positive expression was identified by cell stained with red colour visualized using an inverted optic microscope (Olympus). To examine the potential of differentiation into chondrogenic lineages, mDPSC were cultured with DMEM with high-glucose supplemented with 10% FBS (Cultilab), 100 μg/mL de sodium pyruvate (Sigma), 40 μg/mL proline (Sigma), 50 μg/mL L-ascorbic acid-2-phosphate (Sigma), 1 mg/mL bovine serum albumin (Sigma), 1× insulin-transferrin-selenium plus (Sigma), 100 nM dexamethasone and 10 ng/mL TGFβ3 (Sigma). Control cells were cultured with growth medium. The culture medium was changed twice a week for 21–28 days. To collagen detection, the cells were fixed with paraformaldehyde 4% for 30 min at room temperature, stained with acidic solution of Toluidine Blue (Sigma) for 30 min at room temperature and washed three times with hydrochloric acid 1 N and once with distilled water. The clusters were visualized using an inverted optic microscope.

3. Results

3.1. Morphological features of the mDPCS culture and cytogenetic analysis

Dental pulp stem cells were isolated and expanded *in vitro* from EGFP-transgenic mice. The cells are plastic-adherent and showed rapid expansion and proliferation capacity *in vitro* after isolation. Approximately 80% of the cells proliferated

after 48 h of culture (Fig. 2). A polymorphic morphology was observed in the cell populations obtained. Initially the cells had rounded or fibroblastoid shapes (Fig. 1a). Cells with fusiform (Fig. 1b) and stellate shapes (Fig. 1c) began to appear amongst fibroblastoid cells after 20–28 days of culture. Curiously, in one batch of cells, some elongated cells acquired the contraction capacity (see supplemental material). Proliferative mDPSC showed a normal karyotype in the passages evaluated (Fig. 1d). In only one isolate, tetraploidy was found in 40% of the cells in the sixth passage (data not shown). The formation of cell clusters *in vitro* was also observed (data not shown). More than 90% of the cells expressed GFP (Figs. 2 and 3c). After long term cryopreservation, mDPSC are capable of quickly restarting proliferation in culture, in a manner similar to that of recently isolated cells.

3.2. *Flow cytometric, immunofluorescence, and qualitative transcript analyses*

To investigate the phenotypic characteristics of the mDPSC, cell cultures were analysed using antibodies against several cell surface and intracellular antigens. In the third passage, flow cytometric analysis revealed the expression of cell surface molecules that characterize mesenchymal stem cells, such as CD90, CD73, STRO-1 and Ly6a/Sca-1 (Fig. 2). In contrast, the percentage of hematopoietic cell markers was low (CD117) or undetectable (CD34, CD11b, or CD45) in this passage. The expression of hematopoietic stem cell markers was detected only in the first passage (data not shown). Approximately 80% of the cells were positive for the endothelial cell marker CD31 (Fig. 2). Similar results were observed with cells cultured until the 18th passage (data not shown). Cells were positive for alkaline phosphatase (Fig. 3a) such as observed in the positive control, a culture of embryonic stem cells (Fig. 3b). Curiously, the expression of others embryonic stem cells markers, such as SSEA-1, was strongly positive in mDPSC cultures (Fig. 3d), whereas SSEA-4 and TRA-1-60 markers were not detected by immunofluorescence analysis (Fig. 3e and f). The transcript *ZFP42/Rex-1*, but not *Nanog*, was detected in undifferentiated stem cells by RT-PCR analysis (Fig. 4). Flow cytometry analysis confirmed that approximately 25% of the cells were positive for *Pou5f1/Oct-4* (Fig. 2).

3.3. *Adipogenic, chondrogenic and osteogenic differentiation*

Confluent monolayers of the mDPSC were submitted to conditions known to promote osteogenesis, chondrogenesis and adipogenesis. Control mDPSC were cultured only with growth medium (Fig. 5b, d and f). Cultures of mDPSC in osteoinductive medium had altered cell morphology with a formation of mineral nodules after three weeks of culture. Substantial calcium deposits were seen by Alizarin red-S staining, which localized specifically in the mineral nodules (Fig. 5a). Adipogenic differentiation appeared after two weeks of incubation. Lipid-rich vesicles within the cytoplasm of the cells were evidenced by positive Oil Red O staining (Fig. 5c). In this same time, mDPSC displayed cartilage extracellular matrix differentiation confirmed by the toluidine blue staining (Fig. 5e).

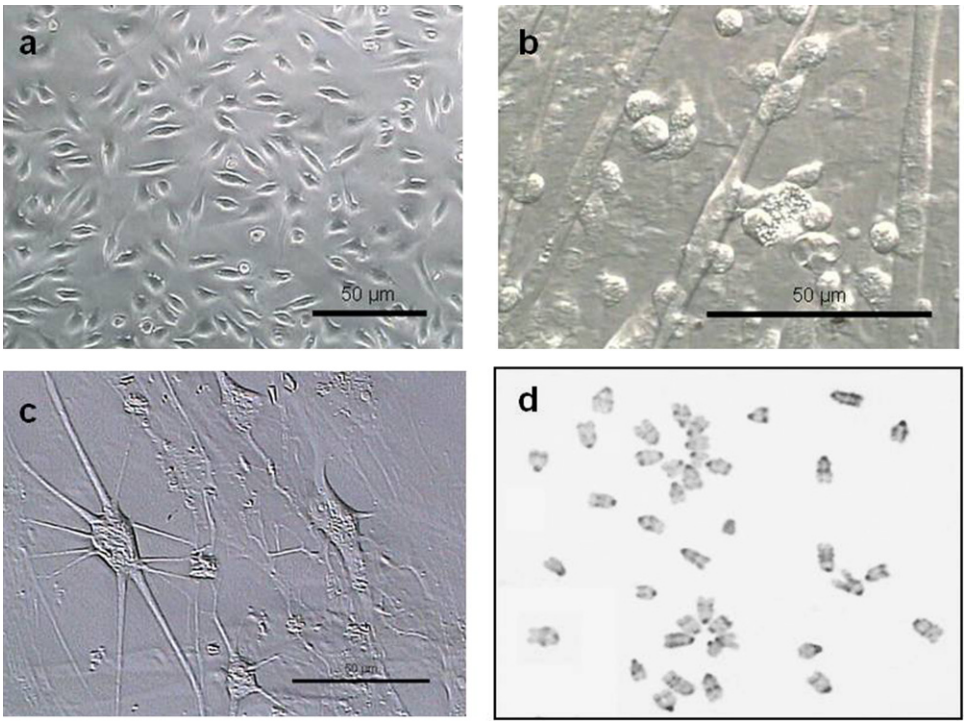

Fig. 1 – Morphologic aspects of mDPSC. Adherent cells cultured *in vitro* present heterogeneity in the morphology. Phase-contrast micrographs were taken on day 5 (a) and day 28 (b and c) of culture. Rounded or fusiform (a), elongated (b) and stellated (c) cell shapes can be observed. Normal karyotype was observed in mDPSC cultured in fifth passage (d).

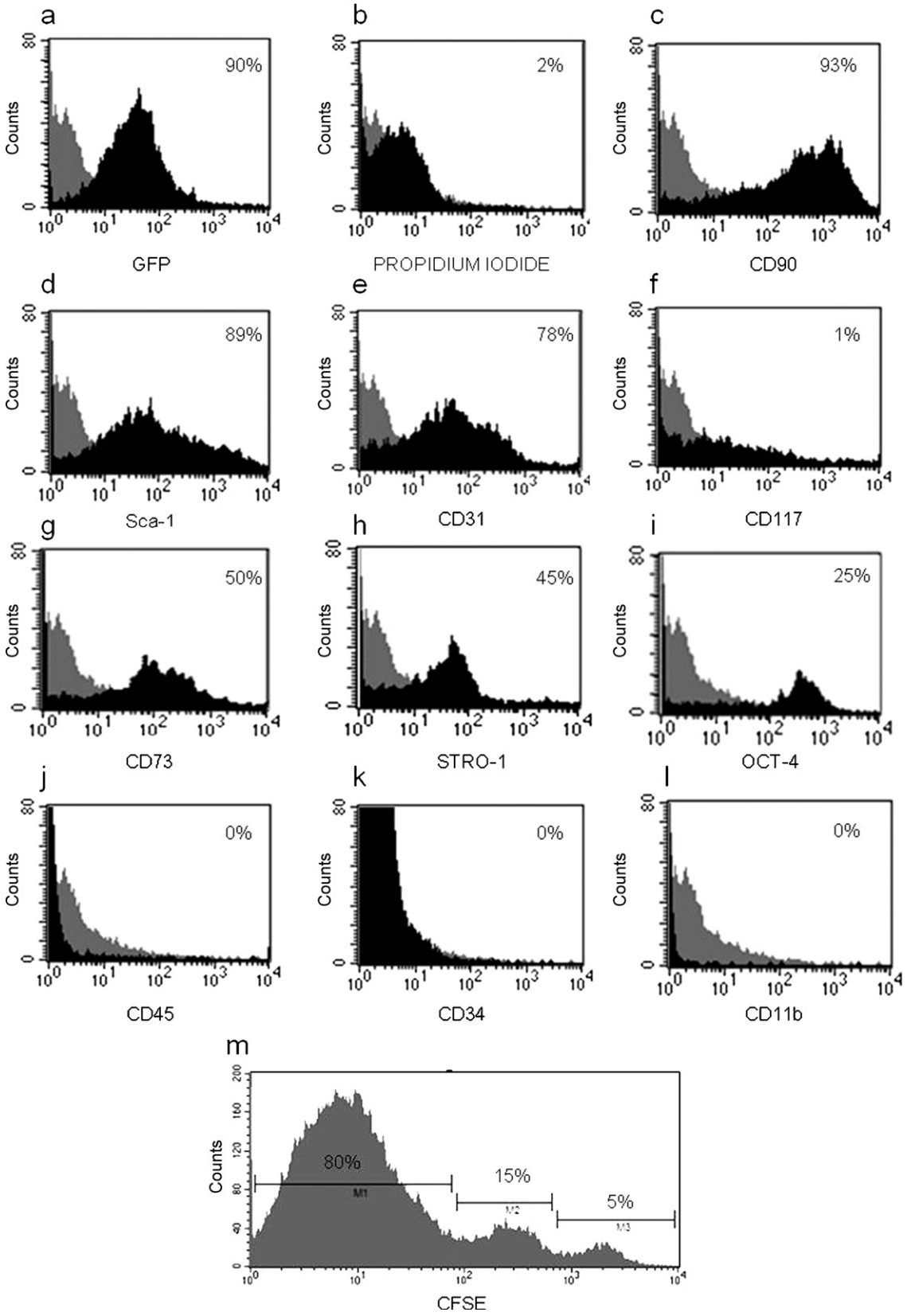

Fig. 2 – Expression of mesenchymal and embryonic, but not hematopoietic stem cells markers in mDPSC. The expression of the surface markers CD73, CD31, STRO-1, CD90, Sca-1, CD45, CD34, CD117, CFSE and oct-4 in mDPSC cultured at the third passage was evaluated by flow cytometry. Histograms show isotype control (grey peaks) versus specific antibody (black peaks) staining, and the percentages of cells positive for the selected molecules (a–l). Proliferative activity of mDPSC by CFSE analysis (m). Results shown were obtained in one representative of three experiments performed.

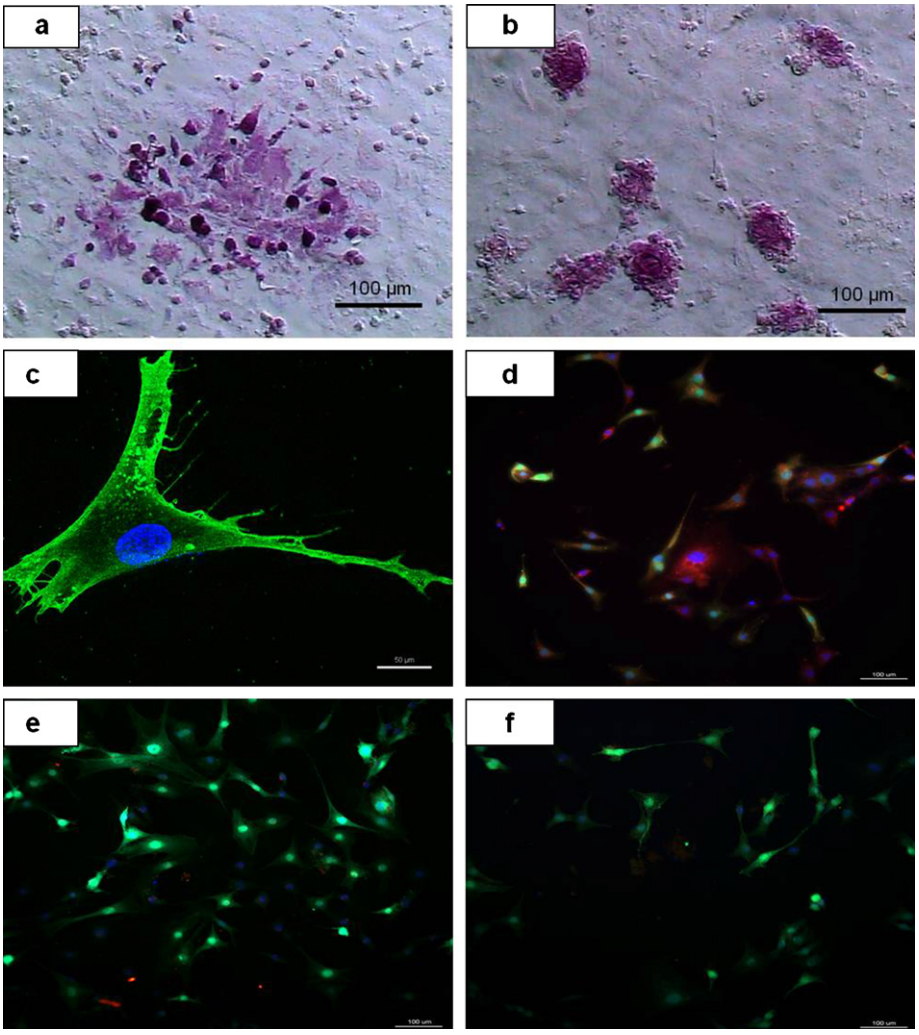

Fig. 3 – Analysis of cell surface markers by cytochemistry and fluorescence. Positivity for alkaline phosphatase staining was evaluated in mDPSC (a) and mouse embryonic stem cells (b), as positive controls. GFP expression in mDPSC (c). The expression of SSEA-1 was positive (red; d), whereas SSEA-4 (e) and TRA-1-60 (f) were not detected. DAPI-stained nuclei are shown in blue. Results shown were obtained in one representative of two experiments performed. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

4. Discussion

Several studies have demonstrated that the human dental pulp is a source of stem cells.[1–7] These cells obtained from deciduous or permanent teeth presents several mesenchymal and embryonic markers, retain the capacity of expansion and differentiation in diverse cell types under chemical defined conditions *in vitro* and repair in vivo.[5–7] Here we isolated, characterized and differentiated stem cells obtained from dental pulp of continuous growth of EGFP transgenic mice. For the immunophenotyping we used similar methodologies employed in the characterization of bone marrow and human dental pulp stem cells (hDPSC),[5,6,18] which have a typical fibroblast-like morphology[5–7] and present no changes in the morphology during 25 passages.[7] In contrast, in the present study we observed morphology alterations of mDPSC according to the culture time. Initially, rounded or fusiform shapes were observed. The elongated and stellate cells began to appear amongst fusiform cells after 28 days of culture. Distinct cell shapes were also observed in other human and murine mesenchymal stem cells, such as bone marrow derived[17–18] and cord blood stem cells.[19]

For clinical applications, an adequate number of cells are necessary and an extensive expansion ex vivo is required. In the third passage, 80% of the mDPSC proliferated after 48 h of culture. This data corroborates with Gronthos et al. data,[5] which demonstrated that approximately 72% of the stem cells obtained from adult human dental pulp proliferate after 24 h of culture. This proliferation index was significantly higher when compared with the stem cells obtained from bone marrow. The authors explained this fact by the extensive fibrous tissue amount in the dental pulp, whereas about 99% of the cells in marrow aspirates are hematopoietic populations.[5] In addition, the stem cells obtained from deciduous dental pulp are more proliferative because of their immature profile.[6]

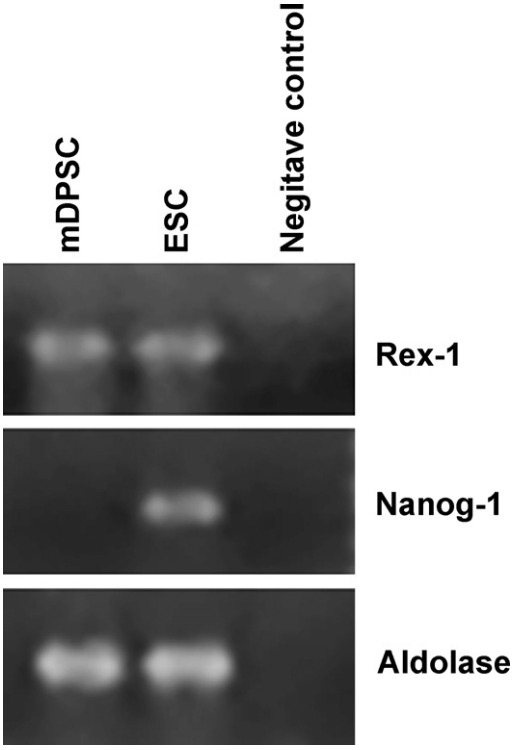

Fig. 4 – Expression of mRNA for pluripotent cell markers. RT-PCR assay for levels of *Nanog*-1 and *ZFP42/Rex*-1 mRNA in mDPSC and USP-1, a mouse embryonic stem cell line was used as a positive control (ESC). Aldolase was used as housekeeping gene.

The proliferative rate can be associated with a progressive chromosomal instability. Malignant transformation of mesenchymal stem cells after expansion in culture has been reported in human and animal models.[20–23] In this case, cytogenetic analysis using G-banding is essential for detecting numerical and structural chromosomal aberrations in stem cell cultures.[24] We verified that mDPSC had a normal karyotype through the fifth passage. In only one isolate, tetraploidy was found in 40% of the cells in the sixth passage. It has been demonstrated that long-term culture and cryopreservation of human embryonic stem cells can lead to a decrease in pluripotency and acquisition of distinct aneuploidies such as a gain of chromosome 17q and an occasional trisomy of chromosome 12 in different passages of the cell cultures.[21,23] Polyploidies and mosaicism produces micronucleation and multinucleations in these cells.[25] In addition, transplanted nude mice with mouse mesenchymal stem cells from bone marrow developed rapidly growing tumours at the injection site after 4 weeks.[26] Miura et al.[27] associated these abnormalities with a gradual increase in telomerase activity and c-myc expression. Time and culture conditions are determinant factors of *in vitro* selection, and it is possible that a clone of cells showing tetraploidy was selectively maintained in mDPSC by cell fusion, for example, resulting in 2n/4n karyotype. This is a relevant aspect to be taken into consideration for future studies using mDPSC in vivo and in experimental models of diseases.

The mDPSC expressed *Pou5f1/Oct-4* and *ZFP42/Rex-1*, transcripts known to be required for self renewal and pluripotency.[28] In contrast, *Nanog* expression was not detected. *Pou5f1/Oct-4* and *Sox2* participates directly of the *Nanog* regulation. However, both *Pou5f1/Oct-4* and *Sox-2* are present in the nuclei of *Nanog*-negative cells of the morula and other precursors, indicating that other molecular signals are required for expression of *Nanog*.[29] We also report that mDPSC express SSEA-1 and alkaline phosphatase, markers of undifferentiated embryonic stem cells. These results confirm the undifferentiated nature of the cells obtained of the mouse dental pulp. Similar results were found in stem cells obtained from human deciduous dental pulp, adipose tissue, bone marrow, heart, and dermis.[7,30] The mesenchymal stem cell markers CD90, STRO-1, Sca-1, and CD73 were also found expressed in mDPSC. In addition, a small percentage of mDPSC expressed CD117. The low frequency of this marker is also observed in the umbilical cord or bone marrow stem cells populations.[31–33] The expression of hematopoietic stem cell markers was detected in the first passage. Several mesenchymal stem cell lines present hematopoietic contaminants in the initial passages of culture.[31] Stem cells obtained from dental pulp of adult rat incisors or isolated from human third molar or deciduous teeth also express a high percentage of mesenchymal cell markers,[5–7,11] such as those observed here in mDPSC. In contrast, a previous report showed that the population of stem cells isolated from dental pulp of erupted murine molars and incisors contains a high percentage of CD45 and CD117 but a low percentage of CD90 and Sca-1 expression. The authors associate this lower expression to the presence of extensive vascularization in the pulp of erupted teeth.[12,34]

An additional property that effectively defines a stem cell is the differentiation potential. The formation of lipid droplets in the cytoplasm, mineral nodules and cartilage extracellular matrix in the mDPSC culture after chemical defined conditions confirmed the adipogenic, osteogenic and chondrogenic differentiation potential, respectively. Not all the cells in mDPSC cultures had the differentiation capability and, in fact, a uniform induced differentiation free of non-responsive cells is very difficult to achieve in mesenchymal stem cell cultures.[35] Interestingly, some elongated cells spontaneously acquired a contractile capacity. In addition of the induced differentiation described in this study, in one isolate it was observed spontaneous differentiation in adipocyte lineage (data not shown). These data indicate the high plasticity of the mDPSC even in the absence of specific stimuli. Stem cells obtained from human or rat dental pulp also exhibit extensive capability of osteogenic, chondrogenic and adipogenic differentiation.[6,7,11] However, Balic and Mina[34] demonstrated that cultures derived from pulps of unerupted and erupted mouse incisors were incapable of differentiating into adipocytes and chondrocytes. The authors suggest that the differentiation in these cell types may be masked by the significant number of osteo/progenitor cells present in the culture which should be investigated in experiments aiming to evaluate the differentiation potential as in vivo transplantation assays. The time of culture, the cell passage or medium used are other factors that may have hampered the differentiation of the cell isolates obtained by Balic and Mina.

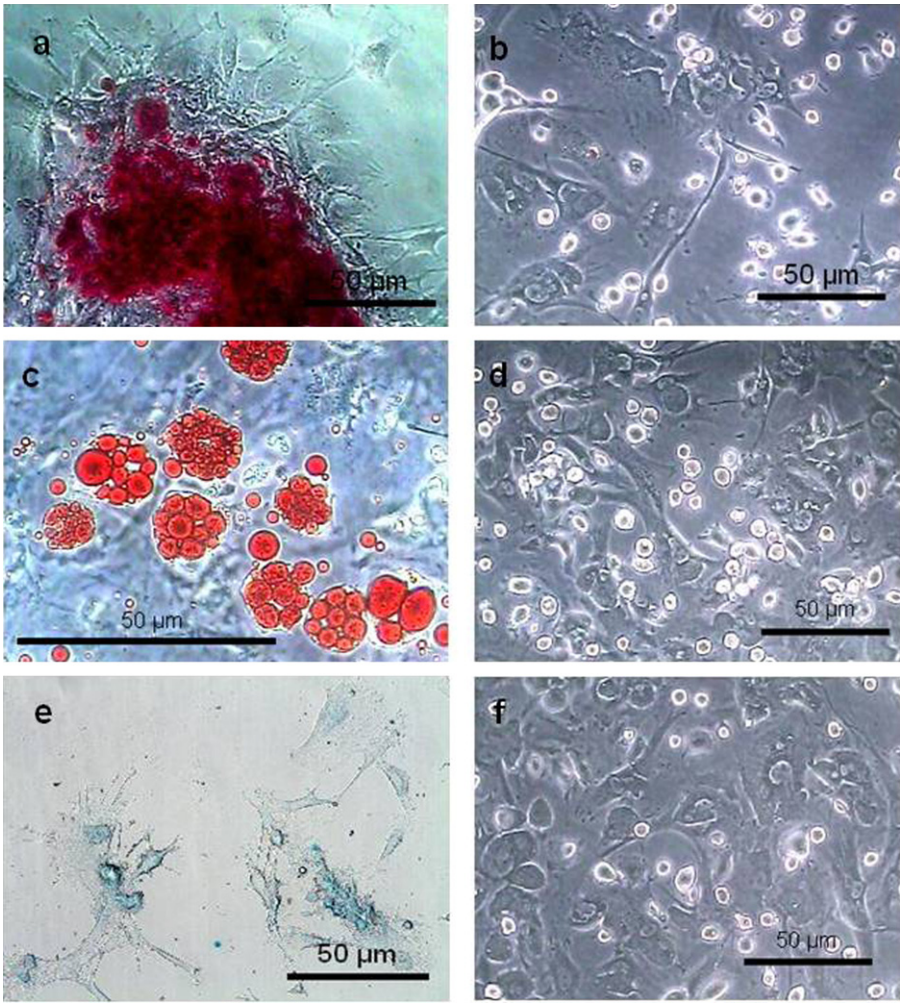

Fig. 5 – Differentiation potential of mDPSC. The mDPSC cells in the third passage were cultured in appropriate media to induce the differentiation into osteogenic (a), adipogenic (c) or chondrogenic (e) lineages. Deposition of a mineralized extracellular matrix (red) was seen after alizarin red staining (a). Lipid vacuoles of differentiated cells are stained with oil red (c). Production of cartilage extracellular matrix confirmed by toluidine blue staining (e). The control cultures in non-inducing medium are shown in b, d and f. Results shown were obtained in one representative of two experiments performed. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

This study provides the description of stem cells obtained from mouse dental pulp, generating cell lines positive for GFP that can be used to track the fate of these cells when injected into different mouse models of disease. The data presented herein demonstrate that mDPSC comprise a morphologically heterogeneous population of cells that exhibit some phenotypic and functional features of both embryonic and mesenchymal stem cells, such as observed in the human dental pulp. The ability to expand and differentiate opens the futures possibilities in the study of the cell therapies in animal models.

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Competing interests: There are no conflicts of interest.

Ethical approval: All of the experimental were approved by the Animal Ethics Committee of the Gonçalo Moniz Research Center-FIOCRUZ, Salvador, Bahia.

Appendix A Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.archoralbio.2011.05.008.

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