ANGIOGENIC POTENTIAL OF HUMAN DENTAL PULP STROMAL (STEM) CELLS

C. MARCHIONNI[1], L. BONSI[1,6], F. ALVIANO[1], G. LANZONI[1], A. DI TULLIO[1], R. COSTA[1], M. MONTANARI[2], P.L. TAZZARI[3], F. RICCI[3], G. PASQUINELLI[4], C. ORRICO[5], A. GROSSI[1], C. PRATI[2] and G.P. BAGNARA[1,6]

[1]Department of Histology, Embryology and Applied Biology, University of Bologna, Bologna; [2]Endodontics Unit, Department of Oral Sciences, University of Bologna, Bologna; [3]Transfusion Medicine Service, S. Orsola Hospital, Bologna; [4]Department of Radiological and Histocytopathological Clinical Sciences, S. Orsola Hospital, Bologna; [5]Department of Specialistic Surgical and Anaesthesiological Sciences, University of Bologna, Bologna; [6]"Giorgio Prodi" Interdepartmental Centre for Cancer Research, University of Bologna, Bologna, Italy

Received February 17, 2009 – Accepted June 23, 2009

Dental pulp is a heterogeneous microenviroment where unipotent progenitor and pluripotent mesenchymal stem cells cohabit. In this study we investigated whether human Dental Pulp Stromal (Stem) Cells (DP-SCs) committed to the angiogenic fate. DP-SCs showed the specific mesenchymal immunophenotypical profile positive for CD29, CD44, CD73, CD105, CD166 and negative for CD14, CD34, CD45, in accordance with that reported for bone marrow-derived SCs. The Oct-4 expression in DP-SCs, evaluated through RT-PCR analysis, increased in relation with the number of the passages in cell culture and decreased after angiogenic induction. In agreement with their multipotency, DP-SCs differentiated toward osteogenic and adipogenic commitments. In angiogenic experiments, differentiation of DP-SCs, through Vascular Endothelial Growth Factor (VEGF) induction, was evaluated by in vitro matrigel assay and by cytometric analysis. Accordingly, endothelial-specific markers like Flt-1 and KDR were basally expressed and they increased after exposure to VEGF together with the occurrence of ICAM-1 and von Willebrand Factor positive cells. In addition, VEGF-induced DP-SCs maintained endothelial cell-like features when cultured in a 3-D fibrin mesh, displaying focal organization into capillary-like structures. The DP-SC angiogenic potential may prove a remarkable tool for novel approaches to developing tissue-engineered vascular grafts which are useful when vascularization of ischemic tissues is required.

Mesenchymal progenitors of human adult dental pulp derive from the most ancestral ectomesenchymal cells responsible for odontogenesis from embryonic to adult life. After birth dental pulp stem cells are able to regenerate adult dental tissues, such as enamel and dentine, damaged by trauma and injury (1-2). The first differentiating process, during tooth formation, starts in the dental papilla where cells differentiate into odontoblasts which are responsible for the synthesis and secretion of primary dentine (3).

Several growth factors and signalling molecules, e.g. Dental Sialoprotein, Transforming Growth

Key words: dental pulp, stem cells, VEGF, angiogenesis

Mailing address: Dr Gian Paolo Bagnara,
Department of Histology,
Embryology and Applied Biology,
University of Bologna,
Via Belmeloro, 8 Bologna 40126, Italy
Tel: ++39 051 2094090 Fax: ++39 051 2094110
e-mail: gianpaolo.bagnara@unibo.it

0394-6320 (2009)
Copyright © by BIOLIFE, s.a.s.
This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.
Unauthorized reproduction may result in financial and other penalties

Factor-beta (TGF beta), Insulin-like Growth Factor (IGF)-I and IGF-II (members of the Bone Morphogenic Protein family) and Fibroblast Growth Factor (4-6), are involved in dentinogenesis during physiological and pathological turnover. The damaged tissue also participates in the repair process, secreting growth factors within the dentine matrix, such as, DSP, TGF beta and BMP-7, and inducing adjacent cells to produce them (5-6). Whenever odontoblast cell survival is compromised, pulp mesenchymal cells are recruited to differentiate into odontoblasts, thus attempting to maintain tissue integrity. Despite the support of mesenchymal stem cells, the physiological reparative capacity of dentine matrix is limited and the efficacy of this response depends on the extent and severity of tissue injury. In addition to odontoblast damage, a necrotic zone associated with partial destruction of the pulp is usually present. This zone involves several types of cells, including vascular endothelial cells (7) which are necessary for proper pulp healing (8).

In a previous report we demonstrated that mesenchymal stromal cells isolated from dental pulp (DP-MSCs) exhibit an immunophenotypic pattern similar to that of bone marrow-derived mesenchymal stem cells (BM-MSCs). However, when compared with BM-MSCs, DP-MSCs show a higher rate of growth and a lower differentiation potential toward adipogenesis; like BM-MSCs, dental pulp derived stem cells are able to suppress stimulated T lymphocyte proliferation, thereby showing an immunomodulatory activity (9). DP-SCs also exhibit a low yield commitment to a myocardial-like lineage *in vitro*, but in the presence of Hyaluronan with Butyric and Retinoic Acid treatment the expression of specific cardiac lineage features increases (10). Like bone marrow stromal tissue, dental pulp is a heterogeneous microenviroment where unipotent progenitors and pluripotent mesenchymal progenitors cohabit (11-12); Shi and Gronthos have demonstrated that DP-derived mesenchymal stromal cells are in a quiescent state in perivascular niches in association with pericytes (13).

In this work we aim to investigate the spontaneous and induced angiogenic ability of DP-SCs stimulated by VEGF through the expression of angiogenic markers and capillary-like structure formation. This ability is thought to be relevant to developing cell-engineered scaffolds (such as fibrin mesh) suitable for ameliorating the trophism of injured tissues.

MATERIALS AND METHODS

DP-SC isolation and culture

Vital human molars were obtained from adult subjects (males, mean age 35 years) during scheduled dental extraction. After extraction, the tooth was immersed in phosphate-buffered solution (PBS) containing antibiotics and antimycotics (penicillin 100 U/mL, streptomycin 100ug/mL, amphotericin B 0.25 ug/mL), in order to avoid any contamination by the germs present in the oral cavity. Teeth were immersed in a chlorhexidine solution (0.2%) in order to decontaminate the tooth surface. Dental crowns were then cut with a sterile diamond bur under constant water cooling. Each tooth was then re-immersed in PBS and transferred in sterile conditions. The pulp was uncovered and extracted. Tissue fragments were mechanically minced, digested with collagenase I 1mg/ml for 30' at 37°C, 5% CO2. The enzymatic digestion was blocked with Fetal Bovine Serum (FBS) and the cell suspension was washed twice by centrifugation in PBS. The pellet was suspended in Dulbecco's Modified Earle's Medium (DMEM) with 10% FBS, antibiotics and antimycotics (penicillin 100 U/mL, streptomycin 100ug/mL, amphotericin B 0.25 ug/mL) and cultured at 37°C, 5% CO2. The cells were expanded with medium changes every 3 days and passaged (harvesting by 500mg/L trypsin - 200mg/L EDTA) up to 15 times.

Immuno-characterization

For flow cytometric analysis, the fibroblast-like cells obtained from dental pulp were harvested at the same point of culture by treatment with 0.25% trypsin-EDTA and incubated with 1 $\mu g/10^6$ cells fitc-conjugated antibodies for 40 minutes at 4°C in the dark. The antibodies used were: SH2 (anti-CD 105), SH3 and SH4 (anti-CD 73) kindly provided by Dr. Mark Pittenger (Osiris Therapeutics, Baltimore, MD, USA) (14); anti-CD29, anti-CD44 and anti-CD166 (Ancell, Bayport, MN, USA); anti-CD14, anti-CD34 and anti-CD45 (Becton Dickinson, San Jose, CA, USA). After washing, cells were analyzed using a Cytomics FC500 Flow Cytometer equipped with two sources (Beckman-Coulter). Results were analyzed using CXP Software (Beckman-Coulter).

Oct-4 expression

Total RNA was extracted from 5×10^5 cells (3^{rd} to 5^{th} passages DP-SCs and HeLa cells as a positive control (15) using TRIzol® Reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). Reverse

transcription reactions were performed in a 20 µl volume with 2 µg of total RNA using Cloned AMV First-Strand cDNA Synthesis Kit® (Invitrogen) following the manufacturer's protocol. The expression of the following mRNAs was investigated: oct-4, Beta actin (control). Primers used in the RT-PCRs were: 1) oct-4: annealing temperature: 60°C, amplicon length 249 bp, sense 5'-CGT GAA GCT GGA GAA GGA GAA GCT G-3', antisense 5'-CAA GGG CCG CAG CTT ACA CAT GTT C-3'; 2) Beta actin: annealing temperature: 55°C, amplicon length 236 bp, sense 5'-GGA CTT CGA GCA AGA GAT GG-3', antisense 5'-AGC ACT GTG TTG GCG TAC AG-3'

The PCR products were visualized on a 1.5% agarose gel stained by ethidium bromide.

Angiogenic differentiation

Confluent cells were cultured in DMEM with 2% FBS and 50 ng/ml VEGF for 7 days, changing the medium every 2 days. DP-SCs cultured in DMEM with 10% FBS for the whole induction period were considered as negative control.

Angiogenesis on Matrigel

Analysis of capillary formation was performed using Matrigel (Becton Dickinson and Co, Franklin Lakes, New Jersey, USA). Fifty µl of gel matrix solution was applied to each well on a 96-well plate and incubated for 1 h at 37°C. Both induced and non-induced cells (1×10^4 cells) were suspended in 50 µl of DMEM, plated onto the gel matrix and incubated at 37°C. Capillary-like structures were observed by optical microscopy after 2, 4 and 8 h and at regular intervals during the following 3 days.

Cytometry analysis of endothelial cell markers

The DP-SCs from the same angiogenic experiment were analyzed, in parallel assays, by flow cytometry using the following antibodies according to the manufacturers' suggestions: Flt-1 (Santa Cruz Biotechnology-USA), KDR (RD System, Minneapolis, USA), CD34 (Beckman-Coulter, Miami, USA), ICAM-1 (Beckman-Coulter, Miami, USA) and vWF (DakoCytomation, Denmark). In order to reveal vWF, the cells were permeabilized by the Intrapep Kit (Beckman-Coulter), incubated with vWF MoAb and subsequently incubated with secondary anti-mouse IgG fitc (DakoCytomation-Denmark).

Data were acquired using a Cytomics FC500 Flow Cytometer equipped with two sources (Beckman-Coulter). Results were analyzed using CXP Software (Beckman-Coulter).

Angiogenesis on fibrin-clot

Analysis of capillary formation was also performed using Fibrin-clot. Ten µl of thrombin was applied to each well on a 24-well plate. Both induced and non-induced cells (25,000 cells/ml) were suspended in a medium containing 0.1% fibrinogen and 10% FBS and seeded on the thrombin layer: the contact of the two materials easily formed a clot which trapped the cells in a three-dimensional position (thrombin and fibrinogen were kindly provided by Kedrion). Capillary-like structures were observed by optical microscopy every 2 hours.

Transmission electron microscopy

For electron microscopy, samples from control and induced DP-SCs entrapped in the fibrin-clot were fixed in 2.5% buffered glutaraldehyde o.n. at 4°C and post-fixed in 1% osmium tetroxide for 1 h at r.t.; following fixation the samples were carefully removed from the well-plate, transferred to plastic tubes, dehydrated in the ascendant series of alcohols, and embedded in epoxy resin. Thin sections were stained with lead citrate and uranyl acetate and observed by a transmission electron Philips 400T microscope.

RESULTS

Isolation and Immunophenotype characterization of DP-SCs

Cells isolated from DP-SC cultures are spindle-shaped and have a fibroblast-like morphology quite similar to that seen in normal BM-MSC cultures (data not shown).

The immunophenotypic profile of DP-SCs proved consistent with that reported in the literature. By flow cytometry DP-SCs expressed surface markers which also characterize BM-hMSCs, and in particular reacted positively with monoclonal antobodies SH2, SH3 and SH4, anti-CD29, anti-CD44, anti-CD166, but were negative for the hematopoietic markers CD14, CD34, CD45 as previously described by Pierdomenico et al. (data not shown).

Oct-4 expression

Oct-4 gene encodes for a relevant regulatory protein involved in the maintenance of self-renewal capacity and undifferentiated state in embryonic stem cells. Stem cell self-renewal is sustained by a critical amount of oct-4, and up- or down-regulation induces cells to follow divergent developmental programmes (15). Through RT-PCR analysis of DP-SCs we observed that the oct-4 expression increased in relation to the number of passages in a cell culture, expression being highest at passage

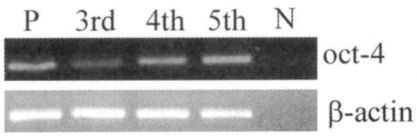

Fig. 1. *RT-PCR analysis of oct-4 expression in DP-SCs. P, positive control (HeLa cells) 3rd, DP-SCs at 3rd passage. 4th, DP-SCs at 4th passage. 5th, DP-SCs at 5th passage. N, negative control (mononuclear cells).*

5 (Fig. 1), indicating that stemness correlates with the increased homogeneity of the cell population in a culture. RNA extracted by HeLa cells (the HeLa cell line) which commonly express this transcription factor (16), was used as a positive control.

Induction of DP-SC differentiation

Differentiation assays towards osteogenesis and adipogenesis were performed for all *in vitro* cultured DP-SC samples. DP-SCs showed osteogenic and, to a lower extent, adipocytic differentiation, but did not differentiate into chondrocytes (data not shown), confirming the results previously described (9).

Angiogenic Differentiation

The Matrigel assay was used to assess the angiogenic potential of DP-SCs. Matrigel is a gelatinous protein mixture simulating the complex extracellular environment found in many connective tissues. Endothelial cell progenitors and terminally differentiated endothelium cultured on Matrigel develop an intricate network of capillary-like structures, which cannot be observed on other substrates (17).

DP-SCs treated with plain culture medium as well as with culture medium containing VEGF were seeded on Matrigel and observed during a 24 h period: they proved able to self-organize into tube-like structures on Matrigel after exposure to angiogenic medium. Unconditioned DP-SCs seeded on top of Matrigel (Fig. 2) did not show any change suggesting vascular development. VEGF stimulated cells showed early morphological changes after 4 h (Fig. 2-B2) and capillary-like structures had clearly developed after 8

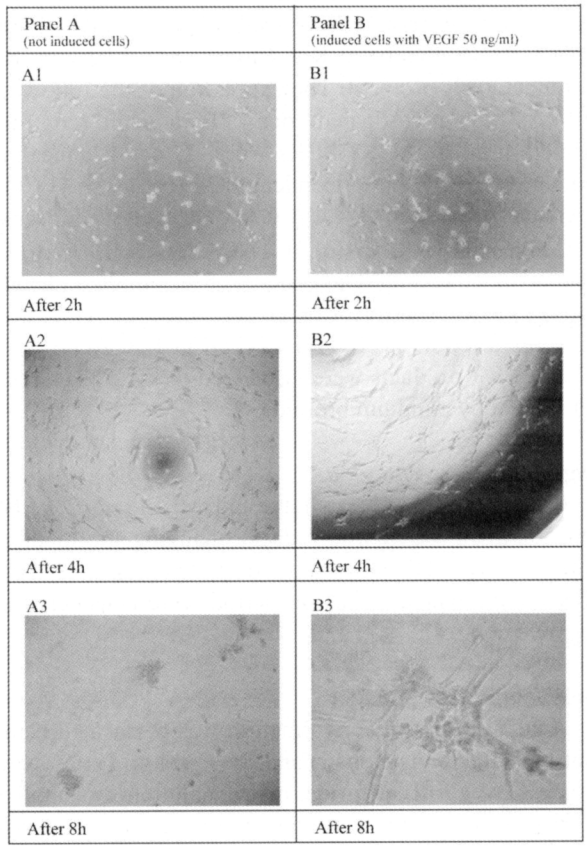

Fig. 2. *Light microscopic analysis of DP-SCs after incubation on Matrigel. (Panel **A**) DP-SCs cultured on semisolid medium after 2 (**A1**), 4 (**A2**) and 8 (**A3**) hours of incubation. (Panel **B**) DP-SCs cultured in angiogenic medium supplemented by VEGF 50 ng/mL after 2 (**B1**), 4 (**B2**) and 8 (**B3**) hours' incubation. A2 and B2 magnification 40x; A1, B1 and A3 magnification 100x; B3 magnification 200x.*

h of culture on Matrigel (Fig. 2-B3).

Oct-4 expression following angiogenic induction

Angiogenic differentiation of DP-SCs induced by VEGF was associated with a decrease in oct-4 expression in RT-PCR (Fig. 3). The stemness potential of dental pulp-derived cells seems to be inversely related to the differentiation and commitment.

Cytometry analysis of endothelial cell markers.

The expression of endothelial cell markers was determined by flow cytometry on controls

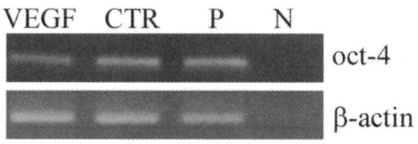

Fig. 3. *Oct-4 expression level in DP-SCs after angiogenic induction. RT-PCR documented oct-4 downregulation as a consequence of VEGF exposure. Samples are as follow: VEGF, DP-SCs treated with Vascular Endhotelial growth factor; CTR, basal growth condition of DP-SCs; P, positive control (HeLa cells); N, reagent control. Beta-actin was used as a house-keeping gene control.*

(Fig. 4, panels A, C, E, G and I) and induced DP-SCs at passage 5 (Fig. 4 Panels B, D,F, H, L). The expression of vWF, a factor of the coagulation cascade constitutively expressed in endothelial cells, increased after VEGF induction. A CD34/vWF dual label plot indicates a sharp shift from a CD34 ^{low} / vWF ^{low} control population (Fig. 4 panel G) to a differentiated CD34 ^{low} / vWF ^{high} population (Fig. 4, panel H); moreover, VEGF promotes vWF cytoplasmic expression in a CD105+ population (Fig 4, panel L). Expression of ICAM-1, an adhesion molecule normally present in low concentrations in endothelial cells and induced by chemokine signals, also increased (Fig. 4, panels E and F).

Analysis of VEGF receptor expression yielded a number of interesting observations. VEGF receptors 1 and 2 (Flt-1 and KDR) were found constitutively expressed in DP-SC and their expression increased following VEGF stimulation. Confirming endothelial differentiation of the cells, flow cytometry analysis revealed an increased expression of Flt-1, KDR, ICAM-1 (Fig. 4 panel B, D, F).

Fibrin Clot cultures: TEM analysis

VEGF-stimulated DP-SCs cultured in a fibrin mesh were able to grow in a 3-D culture system. As shown in Fig. 5 A and B, control DP-SCs when cultured in fibrin mesh displayed a fibroblastlike quiescent morphology. The cells were spindle-shaped with oval nuclei containing marginated chromatin and small compact nucleoli; the cytoplasm contained scant organelles including small mitochondria and cisternae of rough endoplasmic reticulum cisternae; the cell surface was smooth with minimal endocytotic activity; no intercellular junctions or basal lamina-

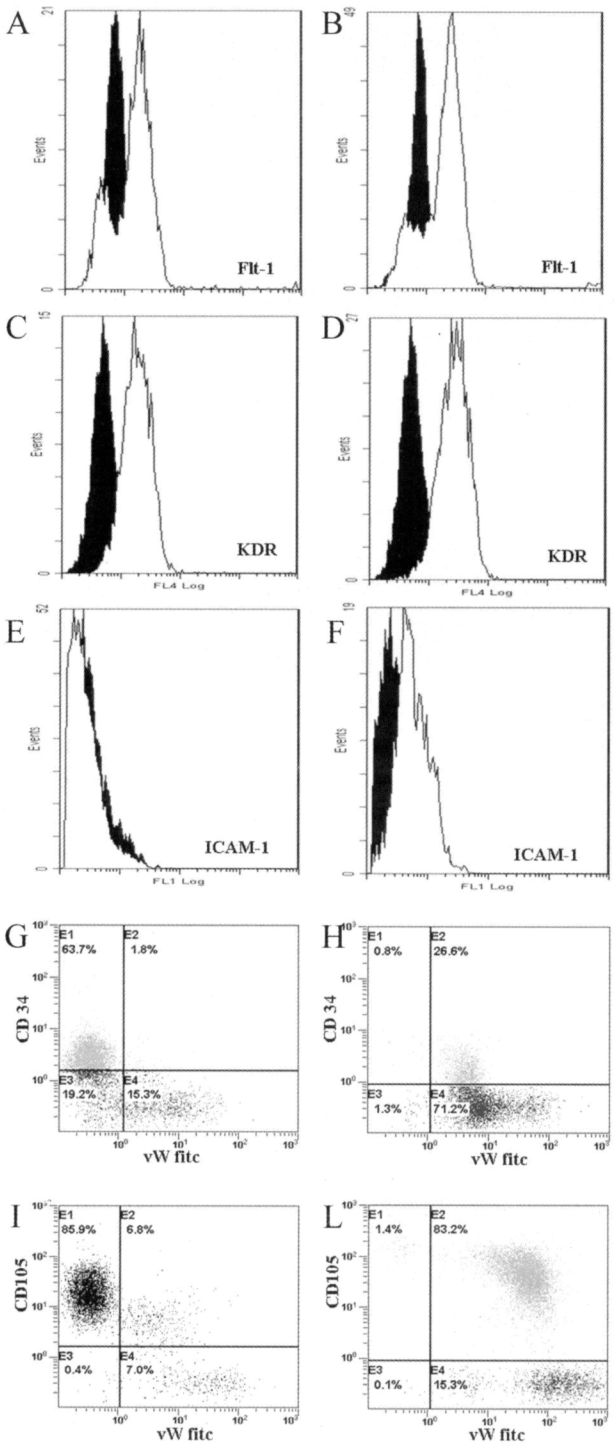

Fig. 4. *Endothelial specific markers after DP-SCs angiogenic differentiation. Flow cytometry analysis for Flt-1, KDR, ICAM-1, CD34, vWF expression in DP-SCs cultured in absence (**A**, **C**, **E**, **G**, **I**) and in presence of VEGF (**B**, **D**, **F**, **H**, **L**). Isotype antibody control in black.*

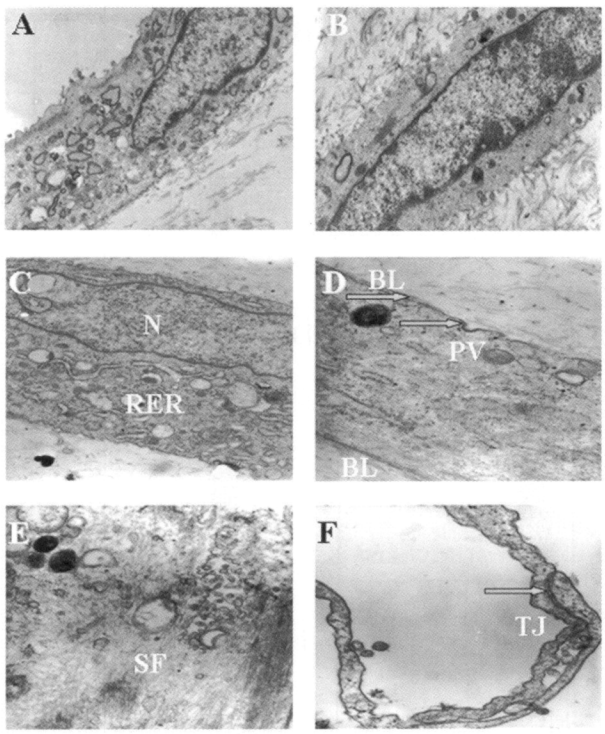

Fig. 5. *Ultrastructural characterization of DP-SCs cultured on fibrin mesh clot. Untreated DP-SCs show fibroblast-like morphology (**A** and **B**). DP-SCs display endothelial features after induction and culture in fibrin clot (**C** and **D**). Particular of cytoskeletric remodeling and endhotelial-like structures appearance on VEGF treated DP-SC (**E** and **F**). N: nucleus; RER: active rough endoplasmic reticulum, PV: pinocytosis vescicles, BL: basal lamina, SF: stress fibers, TJ: tight junctions.*

like material were seen.

In sharp contrast, DP-SCs stimulated with VEGF displayed endothelial cell features when cultured in a fibrin clot (Fig. 5 C and D). The cells become transcriptionally and metabolically active as shown by the presence of chromatin decondensation in the nucleus and prominence of rough endoplasmic reticulum cisternae in the cytoplasm. The ultrastructural evidence of endothelial cell commitment included the presence of coated pits, abundance of pinocytotic vesicles frequently fused together, and invaginating profiles, providing more surface for pinocytic cell activity. Some cells presented extracellular dense matrix material resembling the basal lamina (BL). The occurrence of stress fibers, i.e. contractile acto-myosin structures found in many types of non-muscle cells, including endothelium, indicates cytoskeletric remodeling functional to cell migration within the fibrin substrate. Cells also had long thin cytoplasmic processes which encircled empty areas of the extracellular space. Overall, this cell arrangement mimicked capillary structures, and accordingly tight junctions were seen between the overlapping thin cytoplasmic processes (Fig. 5 E and F).

DISCUSSION

We have previously demonstrated that stromal cells isolated from dental pulp presented a high proliferative profile and immune suppressive activity (9). The stem cell inhibitory activity on the T-lymphocyte response correlates with an increase in soluble HLA-G level promoted by IL10 and this mechanism could be proposed as a marker of stem cell functional ability (18). In addition, osteogenic differentiation was obtained in our laboratory (9) and the osteoblastic-like cell potentiality of dental pulp cells has been confirmed by other authors (19-21). However, DP cells differentiate less towards an adipogenic lineage than those isolated from bone marrow. This different behavior can be attributed to various stages of differentiative commitment by the two populations homing in different niches and/or a different cytokine network involved in each microenvironment. Again, the expression of oct-4, which is associated with an undifferentiated phenotype (22), is also more evident in DP-SCs than in bone marrow-derived stromal cells.

This study also suggests that the number of cell passages is a factor affecting results in cell stimulation experiments, and should be taken into account. Our data demonstrate that the DP cell population was homogeneous and expression of oct-4 was highest at passage 5, both possibly related to stemness capability. Thus, this cell population may be the most suitable for evaluating cell differentiation. In the presence of VEGF, DP-SC could be induced toward endothelial differentiation, as shown by the expression of von Willebrand Factor, a glycoprotein involved in hemostasis and produced constitutively in endothelial cells. Moreover, cytofluorimetric analysis demonstrated an increase in both VEGF receptors KDR and Flt-1 on the membrane surface

of induced cells as well as in ICAM-1 expression. This result is noteworthy because KDR is expressed on adult endothelial cells (23) while Flt-1 appears to be particularly important during vasculogenesis in the embryo (24). Therefore, VEGF-induced cells seem to acquire an angiogenic differentiation ability lasting throughout the embryo development stages. Moreover, vascular tube formation on Matrigel further highlights the endothelial differentiation ability of these cells. When seeded on a matrix able to simulate the basal membrane microenvironment, DP-derived stem cells organized capillary-like structures in the presence of VEGF. It can be speculated that contact with macromolecules present in the extracellular matrix might induce expression of metalloproteases responsible for matrix degradation during the angiogenic process.

TEM observation of DP-SCs, whether induced or not by VEGF and seeded in a fibrin-clot system, showed interesting ultrastructure findings. Non-induced cells maintained a typical fibroblastic morphology like intercellular contacts, while induced cells displayed an ultrastructure similar to the endothelial phenotype, including epithelial morphology, high metabolic activity, tight junctions, endocytosis, coated vescicles and stress fibers. In our experiments, DP-derived induced stem cells expressing vWF did not contain Weibel-Palade bodies, which are usually present in vWF-expressing endothelial cells. We thus conjecture that these cells did not attain complete endothelial differentiation.

Dental pulp is characterized by a very heterogeneous cell population where stem cells reside in the perivascular niche. Our results present evidence that stromal cells isolated by dental pulp, besides other differentiation potentialities and immunosuppressive activity, retain the potential to acquire endothelial characteristics if cultured in appropriate conditions. When cultured in biomaterial, scaffold DP-SCs can reproduce the architecture and microenvironment typical of vessels, and may play a very important role in future regenerative medicine. Again, during myocardial infarction cardiomyocyte loss is associated with lack of blood supply to the area affected and consequently DP-SC ability to differentiate into various cells including cardiomyocytes and endocytes might be taken into account as an angiogenic therapy in autologous reconstruction of the myocardium (10, 25). In this respect it is of interest to note that DP-SCs are able to give rise to vascularized tissue after transplantation *in vivo* (26).

Maintenance of endothelial characteristics in the fibrin clot system might establish an interesting cell vehicle *in vivo* in vascular diseases involving the skin (27) and heart (28), as well as a model for evaluating the role of MSCs in rebuilding the vascular walls after an ischemic injury or trauma.

ACKNOWLEDGEMENTS

These studies were supported by grants from the University Funding and from the Higher Institute of Health via its Stem Cell project to G.P.B.

REFERENCES

1. Tziafas D. The future role of a molecular approach to pulp-dentinal regeneration. Caries Res 2004; 38: 314-20.
2. Bertold PM, Narayanan AS. Periodontal regeneration. In Biology of the Periodontal Connective Tissues. Edited by Quintessence Publishing Co. Ltd. Chicago, 1998; p. 241
3. Ruch JV, Lesot H, B□ue-Kirn C. Odontoblast differentiation. Int J Dev Biol 1995; 39:51-68.
4. Finkelman RD, Mohan S, Jennings JC, Taylor AK, Jepsen S, Baylink DJ. Quantitation of growth factors IGF-I, SGF/IGF-II and TGF-beta in human dentin. J Bone Miner Res 1990; 5:717-22.
5. Smith AJ, Lesot H. Induction and regulation of crown dentinogenesis: embryonic events as a template for dental tissue repair? Crit Rev Oral Biol Med 2001; 12:425-37.
6. Tziafas D, Smith AJ, Lesot H. Designing new treatment strategies in vital pulp therapy. J Dent 2000; 28:77-92.
7. Tran-Hung L, Mathieu S, About I. Role of Human Pulp Fibroblasts in Angiogenesis. J Dent Res 2006; 85:819-23.
8. Roberts-Clark DJ, Smith AJ. Angiogenic growth factors in human dentine matrix. Arch Oral Biol 2000; 45:1013-6.
9. Pierdomenico L, Bonsi L, Calvitti M, et al.

Multipotent mesenchymal stem cells with immunosuppressive activity can be easily isolated from dental pulp. Transplantation 2005; 80:836-42.
10. Ventura C, Cantoni S, Bianchi F, et al. Hyaluronan mixed esters of butyric and retinoic acid drive cardiac and endothelial fate in term placenta human mesenchymal stem cells and enhance cardiac repair in infarcted rat hearts. J Biol Chem 2007; 282:14243-52.
11. Trubiani O, Orsini G, Caputi S, Piatelli A. Adult mesenchymal stem cells in dental research: a new approach for tissue engineering. Int J Immunopathol Pharmacol 2006; 19:451-60.
12. Ballini A, De Frenza G, Cantore S, Papa F, Grano M, Mastrangelo F, Tet□S, Grassi FR. In vitro stem cell cultures from human dental pulp and periodontal ligament: new prospects in dentistry. Int J Immunopathol Pharmacol 2007; 20:9-16.
13. Gronthos S, Cherman N, Gehron Robey P, Shi S. Human Dental Pulp Stem Cells: Characterization and Developmental Potential. In: Adult Stem Cells. Edited by Humana Press Inc NJ: Kursal Turksen, 2004; p. 67.
14. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult mesenchymal stem cells. Science 1999; 284:143-7.
15. Niwa H, Miyazaki J, Smith AG. Quantitative expression of Oct3/4 defines differentiation, dedifferentiation, or self-renewal of ES cells. Nature Genetics 2000; 24:372-6.
16. Tai MH, Chang CC, Kiupel M, Webster JD, Olson LK, Trosko JE. Oct4 expression in adult human stem cells: evidence in support of the stem cell theory of carcinogenesis. Carcinogenesis 2005; 26:495-502.
17. Alviano F, Fossati V, Marchionni C, et al. Term amniotic membrane is a high throughput source for multipotent mesenchymal stem cells with the ability to differentiate into endothelial cells in vitro. BMC Dev Biol 2007; 21:7-11.
18. Rizzo R, Campioni D, Stignani M, et al. A functional role for soluble HLA-G antigens in immune modulation mediated by mesenchymal stromal cells. Cytotherapy 2008; 10:364-75.
19. Nakashima M. Induction of dentine in amputated pulp of dogs by recombinant human bone morphogenetic proteins-2 and -4 with collagen matrix. Arch Oral Biol 1994; 39:1085-9.
20. Shiba H, Fujita T, Doi N, et al. Differential effects of various growth factors and cytokines on the syntheses of DNA, type I collagen, laminin, fibronectin, osteonectin/secreted protein, acidic and rich in cysteine (SPARC), and alkaline phosphatase by human pulp cells in culture. J Cell Physiol 1998; 174:194-205.
21. Alliot-Licht B, Bluteau G, Magne D, Lopez-Cazaux S, Lieubeau B, Daculsi G, Guicheux J. Dexamethasone stimulates differentiation of odontoblast-like cells in human dental pulp cultures. Cell Tissue Res 2005; 321:91-400.
22. Looijenga LH, Stoop H, de Leeuw HP, et al. POU5F1 (OCT3/4) identifies cells with pluripotent potential in human germ cell tumors. Cancer Res 2003; 63: 2244-50.
23. Sato Y, Kanno S, Oda N, Abe M, Ito M, Shitara K, Shibuya M. Properties of two VEGF receptors, Flt-1 and KDR, in signal transduction. Ann NY Acad Sci 2000; 902:201-5.
24. Luttun A, Tjwa M, Carmeliet P. Placental growth factor (PlGF) and its receptor Flt-1 (VEGFR-1): novel therapeutic targets for angiogenic disorders. Ann NY Acad Sci 2002; 979:80-93.
25. Gandia C, Armiñan A, García-Verdugo JM, et al. Human dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction. Stem Cells 2008; 26:638-45.
26. d'Aquino R, Graziano A, Sampaolesi M, Laino G, Pirozzi G, De Rosa A, Papaccio G. Human postnatal dental pulp cells co-differentiate into osteoblasts and endotheliocytes: a pivotal synergy leading to adult bone tissue formation. Cell Death Differ 2007; 14: 1162-71.
27. Nien YD, Han YP, Tawil B, Chan LS, Tuan TL, Garner WL. Fibrinogen inhibits fibroblast-mediated contraction of collagen. Wound Repair Regen 2003; 11:380-5.
28. Christman KL, Vardanian AJ, Fang Q, Sievers RE, Fok HH, Lee RJ. Injectable fibrin scaffold improves cell transplant survival, reduces infarct expansion, and induces neovasculature formation in ischemic myocardium. J Am Coll Cardiol 2004; 44:654-60.