Human dental follicle cells express embryonic, mesenchymal and neural stem cells markers

Rodrigo Lopes de Lima a,*,1, Rosenilde Carvalho de Holanda Afonso b, Vivaldo Moura Neto b, Ana Maria Bolognese a, Marcos Fabio Henriques dos Santos b, Margareth Maria Gomes de Souza a

a Department of Orthodontics, Faculty of Dentistry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil
b Laboratory of Cellular Morphogenesis, Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

ARTICLE INFO

Article history:
Received 26 February 2015
Received in revised form 21 September 2016
Accepted 6 October 2016

Keywords:
Dental stem cells
Human dental follicle
Mesenchymal stem cells
Neural stem cells
Regenerative medicine

ABSTRACT

Objective: This study was conducted to identify and characterize dental follicle stem cells (DFSCs) by analyzing expression of embryonic, mesenchymal and neural stem cells surface markers. Design Dental follicle cells (DFCs) were evaluated by immunocytochemistry using embryonic stem cells markers (OCT4 and SOX2), mesenchymal stem cells (MSCs) markers (Notch1, active Notch1, STRO, CD44, HLA-ABC, CD90), neural stem cells markers (Nestin and β-III-tubulin), neural crest stem cells (NCSCs) markers (p75 and HNK1) and a glial cells marker (GFAP). RT-PCR was performed to identify the expression of OCT4 and NANOG in DFCs and dental follicle tissue.

Results: Immunocytochemistry and RT-PCR analysis revealed that a significant proportion of the DFCs evaluated expressed human embryonic stem cells marker OCT4 (75%) whereas NANOG was weakly expressed. A considerable amount of MSCs (90%) expressed Notch1, STRO, CD44 and HLA-ABC. However, they were weakly positive for CD90. Moreover, it was possible to demonstrate that dental follicle contains a significant proportion of neural stem/progenitors cells, expressing β-III-tubulin (90%) and nestin (70%). Interestingly, immunocytochemistry showed DFCs positive for p75 (50%), HNK1 (<10%) and a small proportion (<20%) of GFAP-positive cells. This is the first study reporting the presence of NCSCs and glial-like cells in the dental follicle.

Conclusions: The results of the present study suggest the occurrence of heterogeneous populations of stem cells, particularly neural stem/progenitor cells, in the dental follicle. Therefore, the human dental follicle might be a promising source of adult stem cells for regenerative purposes.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Mesenchymal stem cells were first identified in aspirates of adult bone marrow. Initially, clonogenic clusters of adherent fibroblastic colony-forming units with the potential to undergo extensive proliferation in vitro and to differentiate into different mesenchymal cell lineages were developed (Friedenstein, Piatetzky-Shapiro, & Petrakova, 1966). Since then, bone marrow had been the most utilized source of MSCs. Nevertheless, there was a need to isolate MSCs from accessible tissues with minimal surgical trauma.

The dental follicle is a loose ectomesenchymally derived connective tissue surrounding the enamel organ and dental papilla of the developing tooth germ. It coordinates the tooth eruption and harbours progenitor cells for the periodontium (Ten Cate, 1997). Dental follicle represents an appealing source of stem/progenitor cells owing to the fact that it is an expendable tissue that can be removed with minimal morbidity (Iked et al., 2006). Through tissue engineering, those stem cells could be exploited to generate more cellular material for tissue repair than could be generated in situ, during the lifetime of an organism (Gronthos, Mankani, Brahim, Robey, & Shi, 2000). This feature confers great potential for the application of such tissue in cell therapy (Iked et al., 2006).

Dental follicle cells have multipotential mesenchymal precursor cell properties after differentiating toward multiple mesenchymal-derived cell types, such as cementoblasts, chondrocytes, adipocytes (Kémoun et al., 2007) and osteoblasts (Morsczeck et al., 2007).

* Corresponding author at: Department of Orthodontics, Faculty of Dentistry, Federal University of Rio de Janeiro, Street Professor Rodolpho Paulo Rocco, 325, Ilha do Fundão, Rio de Janeiro, Brazil.
E-mail address: rololopeslima@gmail.com (R.L.d. Lima).
2. Materials and methods

2.1. Individuals and sample

Six individuals aged between 14 and 16 years were selected to volunteer in the study, according to the following inclusion criteria: a- healthy subjects, with good oral health condition, referred for third molar extraction due to orthodontic reasons and b- presence of impacted third molars with roots in the initial calcification phase, between stages six and seven of tooth development, observed on panoramic radiographs (Noilla, 1960).

The sample included 16 fresh human dental follicles. The region of interest in the dental follicle comprised the part that surrounded the developing roots. Each dental follicle was carefully separated from the roots with aid of a sterile forceps and a scalpel and then washed in phosphate buffered saline (PBS) to remove blood. The tissue remained immersed in solution of chlorhexidine gluconate 0.12% during one minute for bacterial decontamination, and then was washed again in PBS. This study was approved by the Ethics Committee on Human Research of the University Hospital Clementino Fraga Filho Institute of Federal University of Rio de Janeiro (protocol number: 06225113.6.0000.5257) and was conducted according to ethical principles for research involving human subjects of the World Medical Association (Declaration of Helsinki).

2.2. Histological analysis

The follicular tissue was fixed in 4% solution of paraformaldehyde,0.1 M PBS at 4 °C for 48 h. Then, the tissue was dehydrated in ascending series of ethanol and embedded in paraffin. Serial sections of 5 μm were cut in different planes and stained with hematoxylin-eosin (HE) and Gomori trichrome for tissue characterization. Staining with periodic acid-Schiff (PAS) was performed to identify glycoproteins in the extracellular matrix.

2.3. Cell culture experiments

The follicular tissue was cut into small fragments with the aid of sterile scissors and forceps. Then, tissue fragments were enzymatically digested in a solution containing 0.1 U/ml collagenase type II (Sigma-Aldrich) at 37 °C for one hour. Inactivation of collagenase was performed with 1 ml Dulbecco’s Modified Eagle’s Medium (DMEM, Gibco) supplemented with Nutrient Mixture F-12 (DMEM/F12) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Invitrogen Life Technologies). This solution was centrifuged at 1500 rpm for 5 min. The supernatant was discarded and the pellet was resuspended in 1 ml DMEM/F12 with 10% FBS. Minced and digested tissues of dental follicle explants were seeded into T25 flasks in DMEM/F12, 20% FBS, 100 μm l-ascorbic acid phosphate (Sigma-Aldrich), antibiotics/antimycotic (a/a; 100 U/ml penicillin, 0.1 mg/ml streptomycin; Invitrogen Life Technologies), 2 mM L-glutamine (Invitrogen Life Technologies) at 37 °C in 5% CO₂ in a humidified atmosphere. After single cells had attached to the plastic surface, non-adherent cells were removed by changing the medium every 2 days. Plastic adherent cells were propagated until they reached approximately 80% of confluence. During this period, dental follicle cells were examined by phase-contrast microscopy and cells from passages 1 to 5 were used for all experiments. Early passages were used because seems that asymmetric dividing progenitor cells ratify after passing (Morsczeck et al., 2005; Sherley, 2002).

2.4. Immunocytochemistry experiments

Cells were fixed with 4% paraformaldehyde pH 7.4 for 30 min at room temperature and permeabilized with 0.2% Triton X-100 (Reagan) in PBS for 5 min. For blocking, cells were treated with 5% bovine serum albumin (BSA, Gibco) in PBS for 1 h. Cells were incubated with primary antibodies (Table 1) overnight at 4 °C and then with specific secondary antibodies conjugated to Alexa Fluor 488, 546 or 647 (Invitrogen) for 2 h. Nuclear counterstaining was performed with 4’,6-diamidino-2-phenylindole dihydrochloride hydrate (DAPI) at 0.25 μg/μl (Sigma-Aldrich) for 5 min. For negative controls, primary antibodies were omitted and only secondary antibodies were used. Epifluorescence observation and photodocumentation were accomplished using Leica confocal microscope. The proportion of stained cells relative to unstained was expressed in percentage. Fluorescence intensity of markers was classified as low, moderate or high according to Leica

Table 1
Primary antibodies used in immunocytochemistry experiments.

<table>
<thead>
<tr>
<th>Primary antibodies</th>
<th>Antigen</th>
<th>Classification</th>
<th>Species</th>
<th>Sources</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Active Notch1</td>
<td>Active Notch1 receptor</td>
<td>Polyclonal</td>
<td>rabbit</td>
<td>Abcam</td>
<td>1:500</td>
</tr>
<tr>
<td>Anti-CD44</td>
<td>CD44 (HCAM-1)</td>
<td>Monoclonal</td>
<td>mouse</td>
<td>BD-Pharmingen</td>
<td>1:100</td>
</tr>
<tr>
<td>Anti-CD90</td>
<td>CD90 (Thy-1)</td>
<td>Monoclonal</td>
<td>mouse</td>
<td>BD-Pharmingen</td>
<td>1:200</td>
</tr>
<tr>
<td>Anti-Fibronectin</td>
<td>Fibronectin</td>
<td>Polyclonal</td>
<td>rabbit</td>
<td>Sigma</td>
<td>1:200</td>
</tr>
<tr>
<td>Anti-GFAP</td>
<td>Gial fibrillary acidic protein</td>
<td>Polyclonal</td>
<td>DAKO</td>
<td>1:500</td>
<td></td>
</tr>
<tr>
<td>Anti-HLA-ABC</td>
<td>Human leukocyte antigen-ABC</td>
<td>Monoclonal</td>
<td>mouse</td>
<td>BD-Pharmingen</td>
<td>1:200</td>
</tr>
<tr>
<td>Anti-HNK1</td>
<td>Human natural killer-1</td>
<td>Monoclonal</td>
<td>hybridoma</td>
<td>Laboratory</td>
<td>It is not fixed</td>
</tr>
<tr>
<td>Anti-Nestin</td>
<td>Nestin</td>
<td>Monoclonal</td>
<td>mouse</td>
<td>Chemicon</td>
<td>1:100</td>
</tr>
<tr>
<td>Anti-Notch1</td>
<td>Notch1 receptor</td>
<td>Monoclonal</td>
<td>mouse</td>
<td>Santa Cruz</td>
<td>1:50</td>
</tr>
<tr>
<td>Anti-OCT4</td>
<td>Octamer-binding transcription factor 4</td>
<td>Monoclonal</td>
<td>Millsore</td>
<td>1:100</td>
<td></td>
</tr>
<tr>
<td>Anti-p75</td>
<td>Low-affinity nerve growth factor receptor or p75 neurotrophin receptor</td>
<td>Monoclonal</td>
<td>Millsore</td>
<td>1:500</td>
<td></td>
</tr>
<tr>
<td>Anti-Phalloidin</td>
<td>Phalloidin</td>
<td>Monoclonal</td>
<td>mouse</td>
<td>Abcam</td>
<td>1:200</td>
</tr>
<tr>
<td>Anti-SOX2</td>
<td>Sex determining region Y (SRY)</td>
<td>Polyclonal</td>
<td>rabbit</td>
<td>Millsore</td>
<td>1:100</td>
</tr>
<tr>
<td>Anti-STRO1</td>
<td>STRO1 receptor</td>
<td>Monoclonal</td>
<td>mouse</td>
<td>Invitrogen</td>
<td>1:200</td>
</tr>
<tr>
<td>Anti-Vimentin</td>
<td>Vimentin</td>
<td>Monoclonal</td>
<td>mouse</td>
<td>DAKO</td>
<td>1:100</td>
</tr>
<tr>
<td>Anti-α-SMA</td>
<td>α-smooth muscle actin</td>
<td>Monoclonal</td>
<td>mouse</td>
<td>DAKO</td>
<td>1:100</td>
</tr>
<tr>
<td>Anti-β-III-tubulin</td>
<td>β-III-tubulin</td>
<td>Monoclonal</td>
<td>mouse</td>
<td>Promega</td>
<td>1:1000</td>
</tr>
</tbody>
</table>
Application Suite Advanced Fluorescence software (LAS-AF 2.2). To infer the percentage of stained cells and fluorescence intensity was used photomicrographs at the same magnification. All experiments were performed in triplicate and at different times.

2.5. Reverse transcription (RT)-PCR

Total RNA was isolated from 2 g of tissue and 2 × 10⁵ dental follicle cells at passage 1–5 using trizol (Invitrogen), cDNAs were synthesized from 1 μg of total RNA in 20 μl reaction containing 10 × reaction buffer, 1 mmol/l of dinitrophenolphosphate (dNTP) mixture, 1 U/μl RNase inhibitor, 0.25 U/μl reverse transcriptase (Invitrogen). Amplification was performed in a polymerase chain reaction (PCR) thermocycler Veriti (Applied Biosystems) following the reaction profile of: initial denaturation at 95 °C for 3 min, 35 cycles of denaturation at 95 °C for 30 s, annealing at 65 °C for 58 s (amplification of the target gene), extension at 72 °C for 30 s and final extension at 72 °C for 10 min. The following human primers were used for each specific cDNA amplification: OCT4 (forward: 5’ CAGAGGCGAGGGGAGGAGCTAGG 3’, reverse: 5’ CTTCCTCCACAGTGCCCTTAAA 3’), NANOG (forward: 5’ CAGCCCCATCTTC-CACCACTCC 3’, reverse: 5’ CGAAGATCCCGCTGCTTCACC 3’) and GAPDH (forward: 5’ AACGATTTGCTGATATTG 3’, reverse: 5’ GGAGATGCTGATGGATT 3’). 10 μl of each sample was applied to column of agarose gel and horizontal electrophoresis was performed at a current of 100 mA for 45 min. PCR products were photographed under ultraviolet light transilluminator coupled to image analyzer system (L-Pix photo documentation HE – Locus Biotechnology).

3. Results

3.1. Histological analysis and cellular morphology

Histological analysis confirmed that the dental follicle consists of a connective tissue, rich in collagen fibers, covered by thin layer of dental epithelium (Fig. 1a and b). PAS staining showed extracellular matrix rich in glycoproteins (Fig. 1c). At the onset of the cellular culture, DFCs at passage 1 were typical mesenchymal cells with spindle-like morphology (fibroblast-like) (Fig. 1d). After 17 days of culture, DFCs reached approximately 90% of confluence, suggesting a high rate of cellular proliferation (Fig. 1e).

3.2. Immunocytochemistry analysis

Immunocytochemistry evaluation demonstrated that a great proportion (about 75%) of DFCs express moderately transcription factors OCT4 and weakly SOX2 (Fig. 2a and b). Both OCT4 and SOX2 are expressed by human embryonic stem cells and are essential for the establishment and maintenance of undifferentiated pluripotent stem cells. DFCs also exhibited extensive expression of MSCs markers. Notch1 was highly expressed in approximately 90% of cells in culture while active Notch1, a notch1 receptor, was strongly expressed in both nucleus and cytoplasm of approximately 95% of cells (Table 2, Fig. 2c and d). More than 90% of the cultured cells were positive for STRIO and highly positive for CD44 (Fig. 2e and f), HLA-ABC and vimentin (Fig. 3a and b). On the other hand, most of DFCs weakly expressed CD90 (Fig. 3c). Around 70% of DFCs expressed nestin (Fig. 3d), a neural progenitor cells marker. In addition, more than 90% of cells were strongly positive for β-III-tubulin (Table 2), an early neuronal marker. Interestingly, a small proportion of cells (~20%) was positive for GFAP (Fig. 3e and f). In addition, p75 neurotrophin receptor (p75) was identified in 50% of follicular cells, while a small group of cells (~10%) expressed human natural killer-1 (H NK-1) (Fig. 4a and b). Recently studies have characterized HNK-1 and p75 as specific NCSCs markers. Nevertheless, according to the current literature, this is the first time the expression of p75 and HNK1 in DFCs is reported. Elevated proportion (roughly 80%) of α-SMA-positive cells displayed spread morphology with actin stress fibers, similar to the morphology of neural crest-derived myofibroblasts. Overall, DFCs exhibited well-developed cytoskeleton due to the wide conjugation of actin F with phalloidin, and extracellular matrix rich in fibronectin (Fig. 4c–e).

The immunocytochemistry analysis is illustrated in Figs. 2–4. As

Fig. 1. Photomicrograph of human dental follicle showing in (A) follicular tissue exhibiting thin layer of dental epithelium covering the stroma, HE (10×); in (B) is observed stroma rich in collagen fibers, Gomori trichrome (20×); in (C) is demonstrated extracellular matrix rich in glycoproteins, PAS (20×). Phase contrast microscopy showing dental follicle cells with 5 days (D) and 22 days (E) in culture.
previously described, to photomicrographs at the same magnification were used to infer the percentage of satined cells and fluorescence intensity (images not displayed).

3.3. Reverse transcriptase (RT)-PCR

Confirming the immunocytochemistry results, RT-PCR demonstrated that the follicular tissue expresses embryonic stem cells genes, such as OCT4 and NANOG. DFCs displayed a weak expression of NANOG, whereas OCT4 was substantially expressed (Fig. 5). NANOG expression was more pronounced in the follicular tissue than in DFCs, unlike the expression of OCT4 that was similar in both samples.

4. Discussion

The aim of the current study was to propagate undifferentiated cells of human dental follicle and characterize the expression of embryonic, mesenchymal and neural stem cells markers by immunocytochemistry and RT-PCR. The dental follicle is a condensed ectomesenchymal tissue that limits the dental papilla and encapsulates the enamel organ. During the odontogenesis, each tooth has its own follicle (Nanci, 2013). Thus, all teeth in development that need to be extracted are potential sources of dental follicles (Nanci, 2013; Olteanu et al., 2015). The dental follicle, a tissue considered to be neural crest-derived ectomesenchymal cells, contains mesenchymal progenitor cells for the periodontium (Morsczeck et al., 2005). The neural crest comprises a highly pluripotent cell population that migrates towards the first arch to participate in teeth development. Neural crest cells can differentiate into ectodermal and mesodermal cell types (Le Douarin, Creuzet, Couly, & Dupin, 2004).

In our experiments using human dental follicle cells cultures, we identified, by immunocytochemistry, a high proportion of cells (around 75%) that expressed OCT4 and SOX2 in the nucleus and cytoplasm. RT-PCR analysis confirmed OCT4 expression in dental follicle tissue and cells of all passages. Recently, studies related that OCT4, SOX2 and NANOG are weakly expressed in the nucleus of both fresh and cryopreserved dental follicles (Park et al., 2014) while a study reported that DFSCs are negative for OCT4 (Ponnaiyan, 2014). These transcription factors are expressed by
human embryonic stem cells and are essential for the establishment and maintenance of undifferentiated pluripotent stem cells (Okumura-Nakanishi, Saito, Niwa, & Ishikawa, 2005; Rizzino, 2009). The significance of OCT4 and SOX2 expression in a large proportion of cells is unclear. It should be noted in this respect that only a small proportion of amniotic stem cells was positive for OCT4 (Jiang et al., 2002). The lack of OCT4 expression in some cells could be explained by contamination with non-stem cells. Nonetheless, during normal development, OCT4 is expressed in a mosaic pattern in the population of cells that otherwise have equal differentiation potential (Kirchhof et al., 2000). In general, adult stem cells are more restricted in their capacity of differentiation when compared with embryonic stem cells, which can form tumors when implanted in vivo. (Hyndes, Menicanin, Gronthos, & Bartold, 2012). Although approximately 75% of DFSCs express OCT4, these cells are not of originally embryonic cells. Therefore, they have a limited ability to differentiate. More in vivo experimental studies are still necessary to define strict differentiation protocols and to provide and in depth evaluation regarding the behavior of DFSCs, before advancing to the clinical phase. RT-PCR also revealed that DFSCs exhibit weak NANOG expression. As expected, NANOG, a pluripotent cell marker, became downregulated the following passages.

Immunocytochemistry demonstrated that DFSCs express strongly Notch1, a transmembrane protein important in various cell fate decisions during development, and Notch1 receptor. Constitutively active Notch1 reduced the number of the G1 phase cells and accelerated the S phase transition in DFSCs, promoting the G1/S transition (Borghese et al., 2010). G1 phase is a particularly important part of the cell cycle and determines whether a cell remains in the proliferative state or executes other cell fate decisions. Therefore, a shortened G1 phase and an accelerated S-phase transition induced by Notch1 activation may diminish the ability of DFSCs to differentiate, promoting their self-renewal capacity and proliferation (Chen et al., 2013). We identified large proportions of CD44 and STRO1 positive cells (approximately 90%). STRO1 a cell surface protein, characteristic of mesenchymal progenitor cells (Simmons, Gronthos, Zannettino, Ohta, & Graves, 1994), is often used as a marker of dental stem cells (Ulmer, Winkel, Kohorst, & Stiesch, 2010). STRO1 is one the early surface markers of MSCs (Sonoyama, Gronthos, & Shi, 2007) and is necessary for the maintenance of a pool of undifferentiated cells (Miura et al., 2003; Seo et al., 2004). Despite STRO1 is widely expressed by mesenchymal stem/progenitors cells present in the dental follicle (Guo et al., 2012; Guo et al., 2013; Kémoun et al., 2007), a study reported weaker STRO1 expression in human dental follicle cells than in human bone marrow mesenchymal stem cells (BMSCs) (Aonuma et al., 2012). However, one of the factors that can lead to decreased of STRO1 expression is increasing passages (Sonoyama et al., 2007). We observed that most of DFSCs were strongly positive for CD44, a mesenchymal stem/progenitor cells marker. It has been reported that CD44 is equally expressed by DFSCs, BMSCs and MSCs from skin (Park et al., 2012) while a study showed that CD44 expression was higher in DFSCs than BMSCs (Jeon et al., 2011). In the present study, 90% of cultured DFSCs were positive for HLA-ABC, a mesenchymal stem cells marker, that has been identified in periodontal ligament (Kawanabe et al., 2010) and dental pulp (Yazid, Gnanasegaran, Kunasekaran, Govindasamy, & Musa, 2014) cells. To our knowledge, this is the first study demonstrating HLA-ABC positive DFSCs. Most of DFSCs exhibited low expression of CD90. CD90, CD73 and CD105 constitute a minimal phenotypic pattern for the

Fig. 3. Immunocytochemistry of the DFSCs observed by confocal microscopy. The DFSCs exhibiting positive staining for: (A) HLA-ABC, (B) Vimentin, (C) CD90, (D) Nestin, (E) β-III-tubulin and (F) GFAP.
identification of MSCs. The variability in the expression of adult MSCs surface markers could be due to different stages during cell proliferation and cultures (Mafi, Hindocha, Mafi, Griffin, & Khan, 2011).

Immunocytochemistry revealed that DFCs (roughly 70%), express nestin a neural progenitor cells marker, and β-III-tubulin (about 90%), an early neuronal marker, as previously described (Morsczeck et al., 2005; Völlner et al., 2009). It has been reported that DFCs are able to differentiate into neurosphere-like cell clusters when cultured in neurogenic induction medium (Völlner et al., 2009). More recently, it has been demonstrated that DFCs combined with treated dentin matrix differentiate into neuron-like cells (positive for β-III-tubulin and nestin) and are able to regenerate dentin-pulp complex (Jiao et al., 2014). These findings

Fig. 4. Immunocytochemistry of the DFCs observed by confocal microscopy. The DFCs exhibiting positive staining for: (A) p75, (B) HNK1, (C) α-smooth muscle actin, (D) Phalloidin and Active Notch1, and (E) fibronectin.

Fig. 5. RT-PCR analyses of mRNAs in dental follicle tissue and DFCs at different passages. Genes: GAPDH: glyceraldehyde-3-phosphat-dehydrogenase, OCT4: octamer-binding transcription factor 4 and NANOG. C: control, p: passage, T: dental follicle tissue.
suggest that there are neural stem/progenitor cells in the dental follicle at different stages of development. Thus, the human dental follicle can be a promising source of neural stem cells in regenerative therapy. Interestingly, we observed for the first time a small proportion of human DFSCs positive for GFAP (approximately 20%). Previously, a study did not detect GFAP in human DFSCs (Völlner et al., 2009) while other identified weak GFAP expression in murine DFSCs after neural differentiation induction (Ernst, Saugspier, Felthaus, Driemel, & Morsczech, 2009). Conversely, a previous study isolated neural progenitor cells derived from periodontal ligament and differentiated them into a glia-like cell with a high expression of GFAP (Widera et al., 2007). Despite the conflicting results, we believe that the dental follicle cells when subjected to specific stimulus can differentiate into glial-like cells, and therefore, may be a useful tool in tissue engineering field. Moreover, our experiments identified for the first time that DFSCs express moderately p75 (about 50%) and HNK1 (>10%). This suggests the existence of cells with characteristics of undifferentiated neural crest cells in human dental follicle. It has been reported, that a small population (>10%) of human periodontal ligament cells express p75 and HNK1. Those markers have been used to identify NCSCs from human adult tissue (Hara et al., 2014) and human periodontal ligament (Coura et al., 2008; Pelaez, Huang, & Cheung, 2013). Neural crest cells represent a good model in stem cell biology they cells migrate widely and contribute to the formation of diverse tissues during organogenesis. During odontogenesis, neural crest cells migrate to the dental follicle and it seems that some of them remain undifferentiated within that tissue. The present study demonstrated that human dental follicle is a tissue that contains heterogeneous populations of stem cells, which are derived from mesoderm and ectoderm. Embryonic stem cells, mesenchymal, neural progenitor cells and NCSCs were identified. Considering that a large portion of the general population has impacted third molars, the dental follicle could be easily obtained by extraction of impacted teeth. Therefore, the regenerative potentiality of dental stem cells, including DFSCs, has been explored (Rezai-Rad et al., 2015). Moreover, the multiple differentiation potential of DFSCs has demonstrated by experimental studies (Handa et al., 2002; Morsczech et al., 2005; Völlner et al., 2009). Particularly, the strong osteogenic ability of those cells, makes them an attractive type of adult stem cell to repair bone defects or bone loss associated with periodontal disease (Rezai-Rad et al., 2015). Furthermore, the neurogenic potential of some follicular cells indicates that they may be useful for treating neurodegenerative diseases based in cell therapy (Völlner et al., 2009). Further in vivo studies will be necessary to evaluate the regeneration potential of those cells and their possible clinical application.

Conflict of interest

Nothing to declare.

Sources of funding

CAPES, FAPERJ and CNPq.

Ethical approval

This study was approved by the Ethics Committee on Human Research of the University Hospital Clementino Fraga Filho of Federal University of Rio de Janeiro (protocol number: 06225113.6.0000.5257) and was conducted according to ethical principles for research involving human subjects of the World Medical Association (Declaration of Helsinki).

Authors’ contributions

All authors materially participated in the research or article preparation. Below all authors will be listed and the contribution of each of them in the study will be specified.

Rodrigo Lopes de Lima participated in the conception of the idea, study design drafting of the article, acquisition of data, analysis and interpretation of data, and final approval of the article.

Rosenilde Carvalho de Holanda Afonso participated in critical review of the article, data acquisition and final approval of the article.

Vivaldo Moura Neto participated in the design of the study, critical review of the article and final approval of the article.

Ana Maria Bolognese participated in the design of the study, critical review of the article and final approval of the article.

Marcos Fabio Henriques dos Santos participated in critical review of the article, data acquisition and final approval of the article.

Margareth Maria Gomes de Souza participated in the conception of the idea, study design drafting of the article, analysis and interpretation of data, critical review of the article and final approval of the article.

Acknowledgements

The authors gratefully acknowledge the volunteers for participating in this research, maxillofacial surgeons Dr. Albertoino Junior, Dr. Marcelo Galindo and Professor Dr. Paulo José Medeiros, and Fabio Jorge da Silva for technical assistance. This work was supported by Coordination for the Improvement of Higher Level – or Education – Personnel (CAPES, Brazil), Research Support Foundation of the State of Rio de Janeiro (FAPERJ, Brazil) and National Council for Scientific and Technological Development (CNPq, Brazil).

References


