

ISOLATION AND *IN VITRO* DIFFERENTIATION OF CANINE DENTAL PULP STEM CELLS

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Introduction Adult stem cells can be found in different tissues including bone marrow, adipose, epithelial and other tissues. Dental pulp (DP) is composed by ectodermal and mesodermal components. According to our current knowledge, it is considered a source of different types of stem cells. Our group isolated human immature dental pulp stem cells (IDPSCs) from deciduous teeth, which express ES cell markers and present high differentiation potential (Kerkis et al., 2006). Our data have been confirmed by other authors (Huang et al., 2008; Cheng P-H et al., 2008). In order to extend our findings we aimed at the isolation of such cells from dental pulp in canine species. We analyzed the expression of pluripotent stem cell markers and *in vitro* differentiation of canine DP stem cells.

Methods DP (PD1, PD2 and PD3 cells) were obtained from canine deciduous teeth using type Kerr endodontic files. DP tissue explants were gently washed using sterile water and were placed in Petri dishes. After isolation, the cells were maintained in DMEM/F12 + 20% fetal serum bovine. Cells obtained were characterized using specific antibodies for human ES cells, such as anti-Oct3/4, anti-Nanog and anti-Sox2. Cell proliferation rate was evaluated plating 1×10^5 cells/culture flask (25cm²) in triplicate and the cell counting was performed each 3 days during eleven passages. Osteogenic and chondrogenic differentiation were induced following routine protocol for mesenchymal stem cells and evidenced by histological and immunohistochemistry analysis.

Results Mesenchymal-like cells appeared outgrowing from tissue explants after ten days in culture. First cell passage was performed after twelve days and the cells presented continuous exponential growth during eleven passages. The cells presented positive immunostaining with anti-Oct3/4, anti-Nanog and anti-Sox2 antibodies. All studies primary cultures were able to undergo osteogenic after 28 days differentiation presenting mineralization evidenced by von Kossa staining. Osteogenic differentiation also was confirmed by the analysis of expression of sialoprotein and osteocalcin proteins, while undifferentiated cells were negative for antibodies against these markers. To induce chondrogenic differentiation cell pellets were obtained. After 21 days and under appropriate conditions the chondrogenic differentiation was analyzed on tissues sections. The cells, within the sections, presented typical chondroblastic phenotype and extracellular matrix, which showed intensive metachromatic staining with Toluidine blue.

Conclusion Our preliminary data suggest that stem cells can be isolated from canine DP, which present high proliferative rate and differentiation capacity.