COMPARATIVE PHENOTYPIC ASSESSMENT OF PALATAL SUBEPITHELIAL CONNECTIVE TISSUE ISOLATED FROM DOG AND HUMAN

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Abstract

Stem cells isolated from various tissues can self-renew and produce different cell types. Oral cavity may be a valuable source of mesenchymal stem cells (MSCs) that can be isolated and expanded in vitro, providing a unique reservoir of stem cells from accessible tissue resources. The aim of this study was to isolate and assess the multipotent characteristics of MSCs dog and human in order to cover gingival recessions in future with these cells. Human and dog MSCs were obtained from palatal subepithelial connective tissue, expanded and cultured in DMEM/F12 culture medium supplemented with 10% fetal calf serum (FCS) and antibiotics. The cell surface phenotype of the presumed palatal MSCs was characterized at the 4th passage. All flow cytometry measurements were made using a FACS Canto II flow cytometry system (BD Biosciences, San Jose, CA, USA) and analysed using the DIVA program.
Our data confirmed that the isolated and cultivated dog and human MSCs cells have multipotent character based on specific surface antigen expressing (CD44, CD34/45, CD29).

Key words: connective tissue, mesenchymal stem cells, multipotent

INTRODUCTION

The aim of this study was to isolate and assess the multipotent characteristics of MSCs dog and human in order to cover gingival recessions in future with these cells. Mesenchymal stem cells (MSCs) are adult multipotent progenitor cells having the capacity to differentiate into cells of mesenchymal lineage, including bone, fat, and cartilage (Meierlless & Nardi 2009). Oral cavity may be a valuable source of MSCs. Multitudinous types of dental stem cells have been isolated from the human dental pulp (Grontos et al.2000), and dog dental pulp (Wang et al., 2012), exfoliated deciduous teeth (Suchanek et al.2010), apical papilla (Sonoyama et al.2008), tooth germs (Morsczeck et al.2009), palatal periosteum

(Caballero et al.2010), healthy or inflamed periodontal ligament (Park et al.2011). The multipotent cells isolated from these primary sources can self-renew and produce different cell types. Oral cavity may be a valuable source of mesenchymal stem cells (MSCs) that can be isolated and expanded *in vitro*, providing a unique reservoir of stem cells from accessible tissue resources.

MATERIALS AND METHODS

Human and dog MSCs were obtained from palatal subepithelial connective tissue (approximately 2 to 3 mm of the split-thickness connective tissue graft), expanded and cultured in DMEM/F12 culture medium supplemented with 10% foetal calf serum (FCS) and antibiotics. The cell surface phenotype of the presumed palatal MSCs was characterized at the 4th passage. The cultured cells were trypsinized with trypsin-EDTA, washed and then fixed by adding 4% paraformaldehyde for 15 minutes. 1x105/sample were stained at room temperature with isotype control mAbs and then incubated with 3% FBS albumin and centrifuged for 6 minutes at 1800 rpm. The cells were resuspended in 300 to 600 µl of PBS and 2% FBS. All flow cytometry measurements were made using a FACS Canto II flow cytometer (BD Biosciences, San Jose, CA, USA) and analysed using the DIVA program. More than 105 cells were used to detect nonspecific unions or autofluorescence. Data from 10.000 events were recorded.

RESULTS AND DISCUSSIONS

Human tissue (Fig 1.) approximately 1 weeks of culture, some colonies consisting of fibroblast-like cells were observed. These cells were trypsinized and replated for expansion. In order to obtain single cell-derived hPMC clones, cells were serially diluted in 96-well culture plates (BD Biosciences) at a final density of 60 cells/ plate. Cells with homogeneous bipolar morphology were expanded.

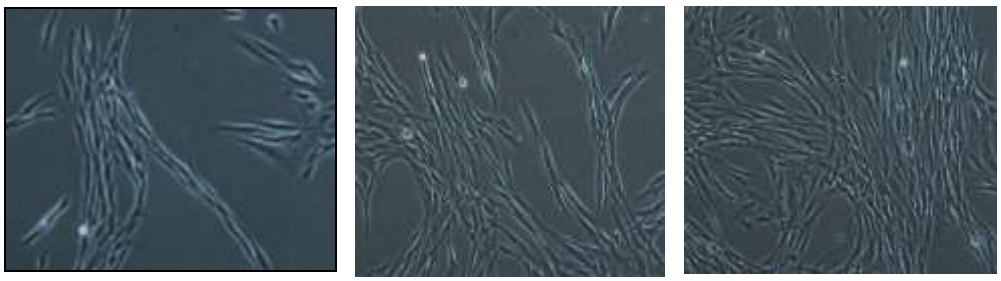

Figure 1 – Human mesenchymal setem cells after isolation and culture

Dog tissue: cells were isolated in explant culture (Fig 2.). After 5 days rapidly proliferative population of cells were isolated.

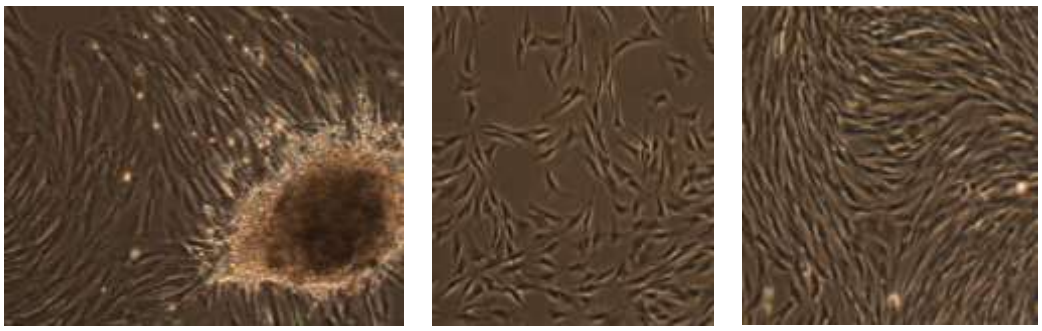

Figure 2 – Dog cells morphology – explant culture

To identify the MSCs, single-cell suspensions were generated from palatal tissue. The ability of palatal-derived cells to form adherent clonogenic cell clusters of fibroblast-like morphology, similar to those recorded for different mesenchymal stem-cell populations, was shown by the formation of about 170-single colonies, generated from 10^4 single cells cultured at low density. These cells firmly attached to the surface of cell-culture plates. The results of the immunophenotypic characterization indicated positivity for CD44, CD29 (Fig 3) and negativity for CD34/45.

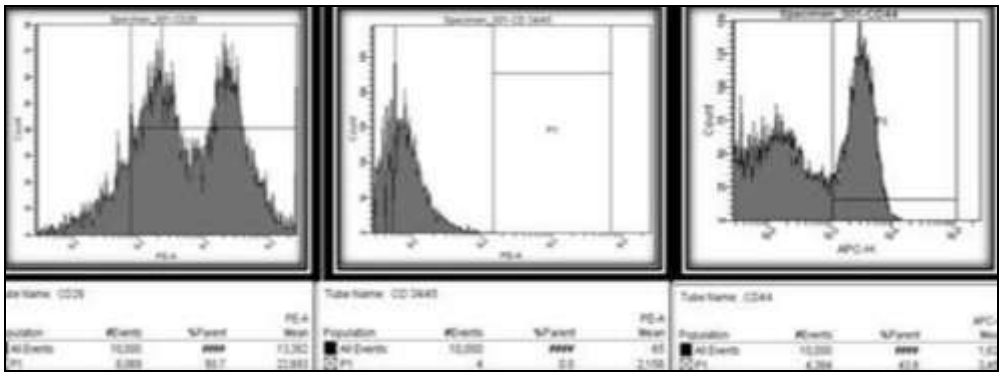

Figure 3 – Phenotypic assessment

CONCLUSIONS

The MSCs obtained (from both sources- human and canine) in this study presented a stable undifferentiated phenotype under normal culture conditions after prolonged cell culture.

MSCs one type of adult stem cell, are easy to isolate, culture, and manipulate in *in vivo* culture. These cells are characterized by high plasticity and can become important cell sources for regenerative therapy.

Our data confirmed that the isolated and cultivated dog and human MSCs cells have multipotent character based on specific surface antigen expressing (CD44, CD34/45, CD29).

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