

# Three dimensional sphere culture system enhances neural crest-related properties of a sub-population of human dental pulp stem cells expressing STRO-1, c-Kit and CD34 markers

Alessandra Pisciotta<sup>1</sup>, Laura Bertoni<sup>1</sup>, Jonathan Mapelli<sup>2</sup>, Albertino Bigiani<sup>2</sup>, Monia Orciani<sup>3</sup>, Marcella La Noce<sup>4</sup>, Anto de Pol<sup>1</sup>, Gianluca Carnevale<sup>1</sup>

<sup>1</sup> Department of Surgery, Medicine, Dentistry and Morphological Sciences with interest in Transplant, Oncology and Regenerative Medicine, University of Modena and Reggio Emilia, Modena, Italy

<sup>2</sup> Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy

<sup>3</sup> Department of Molecular and Clinical Sciences, Università Politecnica delle Marche, Ancona, Italy; <sup>4</sup> Department of Experimental Medicine, Unit of Biotechnology, Medical Histology and Molecular Biology, University of Campania "L. Vanvitelli", Naples, Italy

Human dental pulp, a soft connective tissue contained within the pulp chamber of the tooth, is considered an interesting source of adult stem cells, due to the low-invasive procedures required for cell isolation, high content of stem cells and its peculiar embryological origin from neural crest [1-2]. Based on previous findings from our group, a dental pulp stem cells (hDPSCs) population sorted for the expression of STRO-1, c-Kit and CD34 showed a higher commitment towards neurogenic and glial lineages. Moreover, in standard culture conditions STRO-1+/c-Kit+/CD34+ hDPSCs, at late passages, underwent an arrest in cell proliferation and senescence occurred. To this regard, the aim of the present study was to evaluate the ability of three dimensional sphere structures to preserve the biological and stemness properties of this sub-population. In addition, the ability to differentiate towards neurogenic lineage as well as the expression of Fas ligand were investigated. Our data demonstrated that hDPSCs-derived spheres were able to maintain their fibroblast-like morphology and preserved the expression of the stemness markers and their proliferative capability. At late passages, only few cells derived from spheres were positive for  $\beta$ -Galactosidase activity. Interestingly, the expression of neural crest markers was maintained along the whole culture time and the neurogenic commitment was successfully achieved, as confirmed by confocal immunofluorescence and electrophysiological analyses. The expression of FasL, a key molecule for the modulation of immune response, was observed in undifferentiated hDPSCs derived from sphere culture and, surprisingly, it was maintained even after the neurogenic differentiation was reached, whereas after the induction towards osteogenic and myogenic lineages the expression of FasL significantly decreased ( $P < 0.05$ ). These data demonstrated that 3D spheres obtained from STRO-1+/c-Kit+/CD34+ hDPSCs represent a suitable culture system to preserve the stemness properties and provide a favorable micro-environment for neural crest derived hDPSCs.

## References

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## Keywords

hDPSCs, Neural crest, CD34, FasL