

## Effects of culture system and hypoxia on long-term expansion and differentiation of mesenchymal stem cells derived from periodontal ligament

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Periodontal ligament stem cells (PDLSCs), located in the perivascular space of the periodontium were able to differentiate into periodontal cell types *in vitro* [1]. In this study, we investigated the effect of three different culture media and of low oxygen tension (1%) on the immunophenotype, proliferation rate and osteogenic potential of PDLSCs. This study was the first report to compare the PDLSCs from the same person in different culture systems. PDLSCs were harvested from three healthy third molars and the single-cells suspensions were cultured in the culture media a-MEM, DMEM and a new medium formulation (Enriched Ham's F12 Medium, EHF<sub>M</sub>), respectively. PDLSCs were subcultured ( $4 \times 10^3/\text{cm}^2$ ) until passage 7. The characterization of PDLSCs included FACS, immunofluorescence analysis and cell proliferation assay in both normoxia and hypoxia (1%). After culture in osteogenic medium for 7, 14 and 21 days, osteoblastic differentiation was evaluated by alkaline phosphatase activity, mineralization (alizarin red staining) and gene expression of osteogenic markers. Osteoblastic differentiation was also evaluated under hypoxic conditions. PDLSCs cultured in EHF<sub>M</sub> showed increased proliferation rate and CD73 overexpression compared to cells maintained in a-MEM and DMEM. On the other hand, PDLSCs grown in a-MEM and DMEM showed higher osteogenic differentiation potential compared to EHF<sub>M</sub>. Hypoxia affected both proliferation rate and osteogenic potential. On the basis of these results, we propose a two stages protocol for the osteogenic induction of PDLSCs, in which the early expansion stage could be performed in EHF<sub>M</sub> without loss of cell stemness. Furthermore, the results obtained in the different conditions (normoxia and hypoxia) suggest that oxygen tension plays a critical role in PDLSCs physiology.

### References

- [1] Seo et al. (2004) Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* 364 149-155.

### Keywords

Periodontal ligament stem cells (PDLSCs), Enriched Ham's F12 Medium (EHF<sub>M</sub>), hypoxia, CD73