Reprogramming of Human Gingival and Periodontal Ligament Fibroblasts to Pluripotency with Defined Factors

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Ni-Hung Lin BDS, BScDent(Hons)

Supervisors: Prof Mark Bartold and A/Prof Stan Gronthos

School of Dentistry

The University of Adelaide

DECLARATION

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ABSTRACT

Background: The use of periodontal stem cells with tissue engineering techniques constitutes an attractive strategy for regenerative periodontal therapy. However, technical difficulties of isolating a large quantity of these cells and problem of immune rejection in allogeneic transplantation limit their dental clinical usefulness. Recently, several groups have successfully reprogrammed adult cells to pluripotent cells by retroviral transduction with four genes - Oct3/4, Sox2, Klf4 and cMyc. The resultant induced pluripotent stem (iPS) cells have proliferative and developmental potentials comparable to those of embryonic stem (ES) cells. These cells may represent a good stem cell source for regenerative periodontal therapy. As periodontal tissues are easily accessible, it is hypothesised that periodontal fibroblasts may be an alternative cell source for derivation of iPS cells. **Objective:** The aim of this study was to generate and characterise iPS cells from human adult gingival fibroblasts and periodontal ligament (PDL) fibroblasts. **Methods:** Gingival and PDL tissues from around extracted human adult teeth were collected and digested to obtain single cell suspensions of gingival and PDL fibroblasts. The fibroblasts were lentivirally transduced with mouse receptor for retrovirus mSlc7a1, followed by retroviral transduction with four genes (Oct3/4, Sox2, Klf4 and cMyc). Six days after retroviral transduction, the fibroblasts were re-plated onto mouse embryonic feeders and maintained with daily medium change. At day 25-30, human ES cell-like colonies were harvested for characterisation assays to assess their self-renewal and developmental capacities. **Results:** Human ES cell-like colonies were observed 25 days after transduction. Cells from these colonies were morphologically similar to human ES cells, expressed ES cell genes assayed by immunostaining and real-time reverse-transcription polymerase chain reaction, showed silencing of exogenous retroviral genes and displayed a normal karyotype. *In vitro*, these cells formed embryoid bodies with down-regulated expression of ES cell genes

and up-regulated expression of ectodermal, mesodermal and endodermal markers. **Conclusion:** iPS-like cells can be generated from human adult gingival and PDL fibroblasts, and gingival fibroblasts can represent an easily accessible source of cells to derive individual-specific iPS cells for regenerative periodontal therapy.

PUBLICATIONS

Published Papers

Lin NH, Gronthos S, Bartold PM. Stem cells and future periodontal regeneration. Periodontol 2000. 2009; 51: 239-51.

Wada N, Wang B, Lin NH, Laslett A, Gronthos S, Bartold PM. Derivation of human induced pluripotent stem cells from periodontal ligament fibroblasts and gingival fibroblasts. Stem Cells Dev (in preparation).

Published abstracts

Wada N, Lin NH, Gronthos S, Bartold PM. The establishment of human induced pluripotent stem (iPS) cell lines from periodontal ligament cells and gingival fibroblasts. Adelaide: Colgate Australian Clinical Dental Research Centre (CACDRC) Research Day 2009 (Abstract).

Lin NH, Wada N, Wang B, Laslett A, Gronthos S, Bartold PM. Derivation of human induced pluripotent stem cells from periodontal ligament fibroblasts and gingival fibroblasts. Adelaide: The Robinson Institute, Centre for Stem Cell Research Annual Meeting 2009 (Abstract). Winner of Runner-up Poster prize ($500).

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