Stem-like cells obtained by dedifferentiation of gingival fibroblasts

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INTRODUCTION

Loss of dental structures due to cavities, trauma or periodontal disease represent the main oral health problem worldwide, with an incidence of practically 100%. Although artificial substitutes for dental tissues are available, they are only able to partially replace biological functions, a situation that regenerative dentistry (RD) aims to overcome. The RD has been explored by two methodologies: (1) collection, selection, and differentiation of stem cells and (2) reproduction of dental embryogenesis. However, there are important limitations, especially obtaining the desired cells and associated ethical and rejection issues. This way, innovative strategies to obtain stem-like cells for use in RD is an imperative need. The use of gingival fibroblasts presents several advantages: easy collection in a simple surgical procedure in the dental office, rapid regeneration and without associated ethical problems.

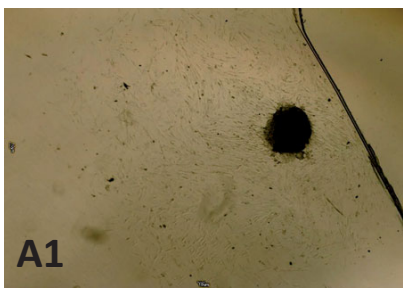
AIM

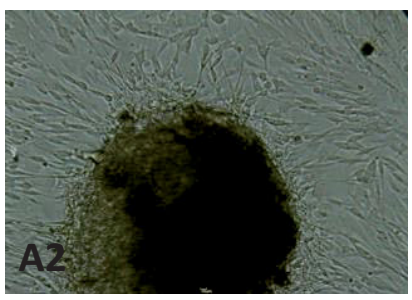
The aim of this work is to apply a methodology of cellular dedifferentiation into gingival fibroblasts, hence to obtain stem-like cells, which in turn can be differentiated into cells of interest, for use in Regenerative Dentistry.

METHODOLOGY AND RESULTS

A. Murine gingival fibroblast cell cultures

Balb/C mouse, 8 weeks old were used. Briefly, gingival tissue is collected from the animal, fragmented in 1mm^3 pieces and digested with trypsin and collagenase type I. The fragments are cultured and when confluence is achieved, cells are used in experiments.



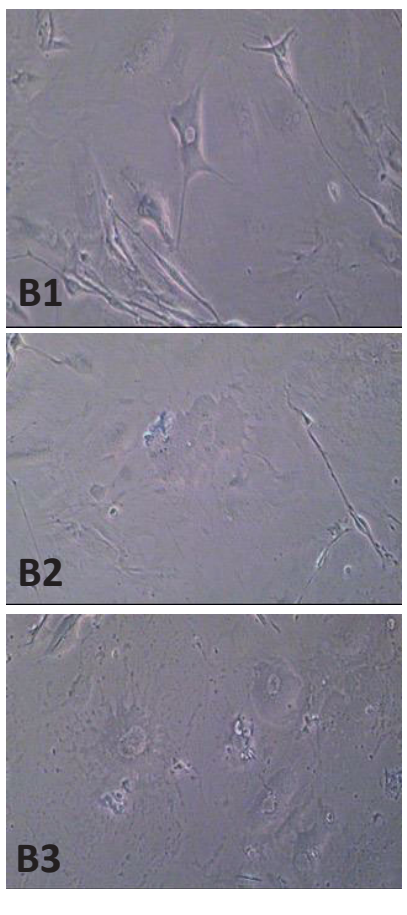


Successful established primary cultures of murine gingival fibroblasts trough the explant methodology

Magnification of 10x (**A1**) and 100x (**A2**).

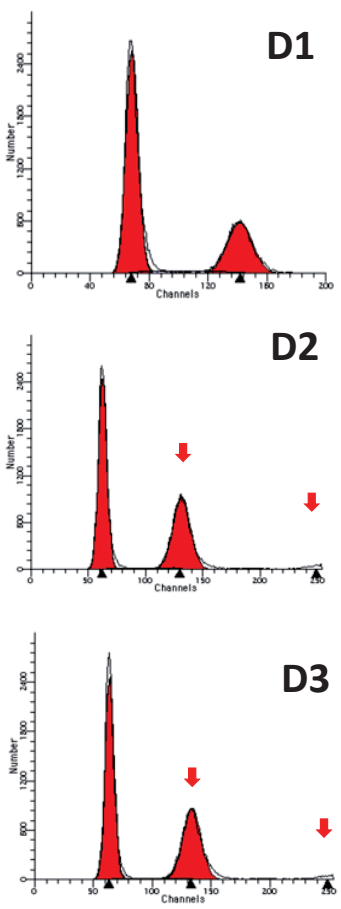
B.C.D.E.F. Gingival Fibroblasts Deddiferentiation

Deddiferentiation was promoted with an agent (DA). A screening of concentrations was performed (from 1 to 5µM) and mode of incubation (with and without growth media changes), to verify which concentration induce the formation of a tetraploid population as described, maintaining cell viability .



DA promotes increase in cell size and morphological changes in gingival cells and cell death in some cells

Optical microscopy images of gingival fibroblasts (**B**) and after crystal violet staining **(C)**: **B1/C1**: control; **B2/C2**: after DA 5µM 1x; **B3/C3**: after DA 5µM 4x.



DA lead to G2/M increase and the appearance of a population with double DNA amount, probably due to tetraploid formation

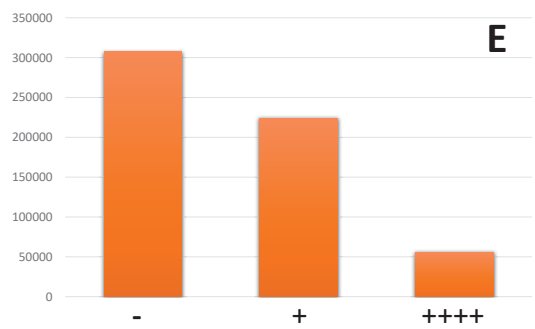
Cell cycle analysis. **D1:** control; **D2**: after DA 5µM 1x; **D3**: after DA 5µM 4x.

DISCUSSION AND CONCLUSION

These preliminary results point that the DA succeeds in promoting gingival fibroblast dedifferentiation, with morphological and genetic changes compatible with stem-like phenotype.

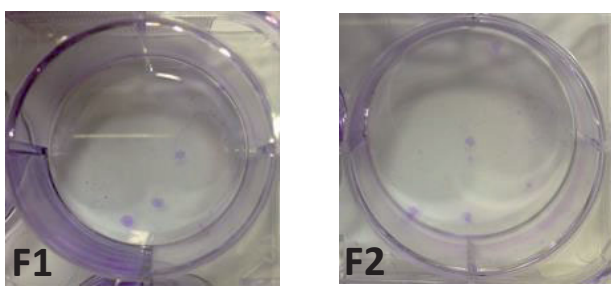
CLINICAL IMPLICATIONS

Stem-like cells obtained by gingival fibroblasts dedifferentiation, can be used in the future in dentistry regenerative procedures.



DA promotes a decrease in cell proliferation of gingival cells

Cell proliferation (**-**: control, **+**: after DA 5µM 1x and**+++**: after DA 5µM 4x).



DA promotes a decrease in clonogenic efficiency of gingival cells

Clonogenic efficiency (staining with crystal violet): **F1**: control: **F2**: after DA 5µM 4x.

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