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DENTAL STEM CELLS DIFFERENTIATING INTO OSTEOBLASTS REPRESENTING A PERFECT CELL SOURCE FOR BONE REGENERATION

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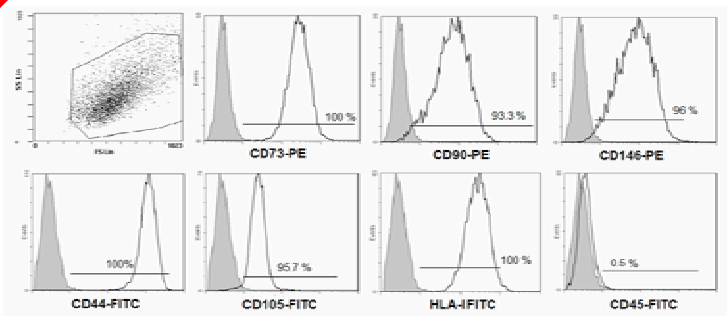
INTRODUCTION

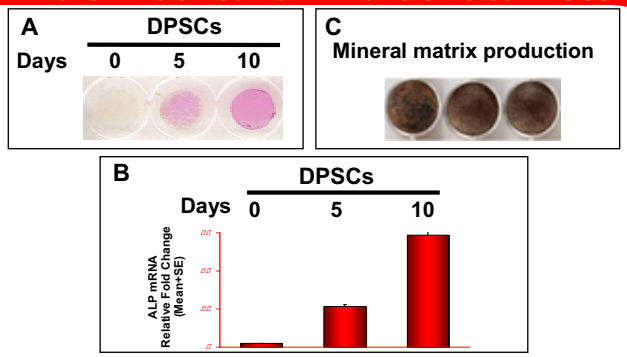
Stem cells are defined as clonogenic cells capable of self-renewal and multi-lineage differentiation. A population of these cells has been identified in human dental pulp. Dental Pulp Stem Cells (DPSCs) were found in adults teeth and have been shown to differentiate, under particular conditions, into various cell types of the mesenchymal tissues. In this work we studied the immunophenotype of DPSCs by flow cytometric analysis. DPSCs were then cultured in osteogenic medium and the osteoblastic markers were analyzed by histochemical method and real-time PCR. In particular, the typical osteoblast markers such as Alkaline Phosphatase (ALP), Collagen type-I (Coll-I), RUNX2, Bone Morphogenetic Protein 2 (BMP-2) and Osteopontin (OPN),as well as mineralized matrix production were detected.

Mesenchymal markers are expressed by DPSCs



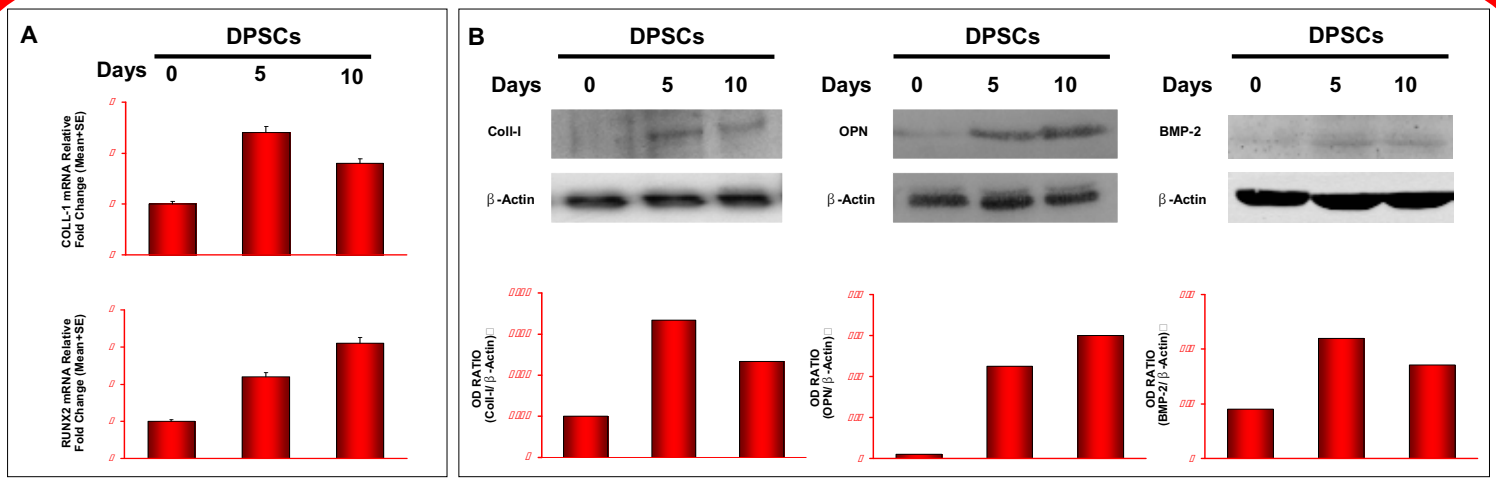
Phenotypes of DPSCs. The expression of the indicated mesenchymal stem cell markers on DPSCs was analyzed using flow cytometry. Results from one representative DPSC culture are shown. The grey histograms signify staining with isotype controls and the white histograms represent staining with the specified surface marker antibody.

ALP and mineralized matrix in differentiated DPSCs



Alkaline phosphatase evaluation and mineralized matrix production. Histochemical staining for ALP in DPSCs incubated or not (day 0) in osteogenic medium for 5 and 10 days (A). The expression of ALP was also analyzed under the same experimental conditions by RT-PCR (B). Mineralized nodules formation by three representative samples of DPSCs incubated for 30 days in osteogenic medium (C).

Osteoblast markers are expressed by DPSCs cultured in osteogenic medium



Collagen type I, RUNX2, Osteopontin and Bone Morphogenetic Protein 2 expression. RT-PCR(A) analyses show the expression of Coll-I and RUNX2 at day 0 and after 5 and 10 days incubation of DPSCs in osteogenic medium; Western-blot analysis (B) shows Coll-I, OPN and BMP-2 expression in DPSCs incubated or not (day 0) in osteogenic medium for 5 and 10 days; the OD ratio of these proteins versus β-Actin is reported in the graph.

CONCLUSION

We demonstrated that DPSCs successfully differentiated into osteoblast-like cells, producing mineralized matrix nodules and expressing the typical osteoblastic markers, such as Alkaline Phosphatase , Collagen type I, RUNX2, Osteopontin and BMP-2. This study suggests that DPSCs, differentiating into osteoblasts, represent a perfect source of cell for bone regeneration.