

CHARACTERIZATION OF MINERALIZED-TISSUE-FORMING CELLS IN CULTURE OF HUMAN PERIODONTAL CELLS STIMULATED BY ENAMEL MATRIX DERIVATIVES USING FLOW CYTOMETRY

S. Skarsvik¹, J. Ekberg¹, [S. Lemoult¹](#), [R. Seibl²](#), [M. Dard²](#) and [P. Boissy¹](#)

¹ *Cell Biology Department, [Straumann/Biora AB](#), Medeon Science Park, Malmö, SE*

² *PreClinical Research, [Institute Straumann AG](#), Basel, CH*

INTRODUCTION: Emdogain®, which contains Enamel Matrix Derivatives (EMD), is used for stimulating the regeneration of periodontal tissues lost during periodontitis [1]. This process is initiated in the remaining periodontal ligament (PDL), a non-mineralized connective tissue that harbours progenitors for cementum and bone. Several in vitro studies have reported that EMD stimulates proliferation of PDL cells and induces tissue mineralization [2-3]. However little is known about the responsive cell fraction, its phenotype and whether these cells turn into cementoblasts or osteoblasts.

Consequently, in the present work, flow cytometry has been implemented in order to study PDL cell response to EMD. This technique has the unique advantage to analyse cells individually for different parameters and can discriminate between subpopulations of cells.

METHODS: The distribution and expression of bone/liver/kidney isoform of alkaline phosphatase (ALP) and osteocalcin (OC), two markers associated with tissue mineralization as well as STRO-1, a mesenchymal stem cell marker recently identified on PDL cells [4] have been analyzed. PDL cells were treated with EMD for 7 days in absence or presence of 1.25(OH)2VitaminD3 (VitD3) used as osteoinductive factor.

RESULTS: It has been found that EMD strongly upregulated ALP expression in PDL cells costimulated with VitD3 in a dose-response manner. ALP expression was only slightly up-regulated by EMD or VitD3 alone suggesting that VitD3 and EMD had a synergistic effect on this marker. Furthermore, these results corroborated well ALP activity measured in an enzymatic assay. We also succeeded in detecting OC and its expression showed to be up regulated by EMD in a dose dependent manner but independently of the hormonal treatment. STRO-1 was detected on

15-20% PDL cells in subconfluent cell cultures and its expression was up-regulated by VitD3 alone. Costaining for STRO-1 and ALP showed that there was an inverse association between the two markers in which the up-regulation of ALP expression induced by EMD was associated with a strong decrease of STRO-1 expression.

DISCUSSION & CONCLUSIONS: It has been shown for the first time that the differentiation of PDL cells into mineralized-tissue forming cells can be analyzed by flow cytometry and that this technique is a complementary tool to traditional methods. We have found that the treatment of PDL cells with EMD stimulates the expression of ALP and OC in a dose-dependent manner while it down regulates the stem cell marker STRO-1. This technique opens new perspectives of research on periodontal regeneration since it is a unique tool to characterize further subpopulations of progenitors present in the PDL tissue, their response to EMD and their differentiation into bone and cementum forming cells.

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