“Differentiation and Characterization of Epithelial Cells and Fibroblasts from Stem Cell from Human Exfoliated Deciduous Teeth (SHED)”

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Oral mucosa is a specialized type of tissue that lines the oral cavity. In regenerative dentistry, the development of three-dimensional (3D) oral mucosa model using tissue engineering approach is very significant for *in vitro* studies of mucosal irritation, biocompatibility and diseases as well as clinical applications. This model has been developed with the use of oral epithelial cells and fibroblasts cultured on a scaffold. However, the epithelial cells and fibroblasts which were commonly derived from oral biopsies actually could bring about problem of cost, patients, surgical procedures and time constraint. Hence, in order to overcome these problems, the present study focused on the differentiation of stem cells from human exfoliated deciduous teeth (SHED) into epithelial and fibroblast-like cells to be used subsequently in future development of 3D oral mucosa model. The differentiation of epithelial and fibroblast-like cells from SHED was carried out with the involvement of growth factors, i.e. four multiple combinations of growth factors (keratinocyte growth factor, epidermal growth factor, hepatocyte growth factor and insulin-like growth factor II) were used for epithelial differentiation, whereas a specific human recombinant connective tissue growth factor was used for fibroblastic differentiation. The differentiated cells were then characterized by (i) morphological observation, (ii) proliferation rate, (iii) gene expression quantification using reverse transcription-polymerase chain reaction (RT-PCR), (iv) immunofluorescence staining and (v) flow cytometry analysis for detection of E-cadherin and FSP-1, respectively. The commercial human keratinocytes and human gingival fibroblasts served as positive control in this study to ensure the cells differentiated from SHED were epithelial cells and fibroblasts. The results showed that SHED derived-epithelial cells and fibroblasts were successfully characterized i.e. (i) having similar appearance as the positive control cells (ii) significant proliferation rate between epithelial-like cells, fibroblast-like cells and SHED, (iii) high expression of epithelial and fibroblast-associated markers in qRT-PCR analysis and (iv) positive staining against E-cadherin and FSP-1 in immunofluorescence staining and flow cytometry analysis, respectively. The same expression patterns were found in the commercial positive control cells. SHED as negative control cells showed less/negative signal, hence ascertained the validity of the staining. Taken together, the protocol adopted suggests growth factors used in this study to be appropriate inducers in the successful differentiation of SHED into epithelial and fibroblast-like cells.

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