

Tissue engineering for the development of three-dimensional *in vitro* models of human mucosae

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The recent progress in the biomaterials field and the need for novel 3D cell culture models has led us to develop a new model, the 3D mucosal outgrowth, with advanced characteristics: the simultaneous presence of two differentiated cytotypes (a polarized epithelium above a fibroblastic layer), a complex extracellular matrix (ECM) and the possibility for long-term exposures. This model can be used to grow different human mucosae *in vitro*, with two types of support scaffolds, a porous polyethylene terephthalate (PET) membrane, for which the model was initially developed, and a poly-L-lactide (PLLA) membrane that was later adapted to the model due to its biodegradability. A fragment of the tissue of interest is placed on the membrane and embedded within a synthetic extra cellular matrix; during the culture period, cells start to grow outwards from the central biopsy and form a new mucosa. Outgrowths grown on PET and PLLA were characterised morphologically by electron microscopy, and immunotyped by immunofluorescence and immunohistochemistry. When grown on PET, all tested mucosae (bronchial, oral and colorectal) showed good differentiation of the relevant cytotypes and proper organization of the ECM. For this reason, they were used for functional studies. Outgrowths developed on PLLA instead, showed a lower degree of cell differentiation and a tendency to form tubular growths that stained positively for endothelial markers. Further evaluations are needed to verify the possibility to use PLLA as a support scaffold for this model.

Keywords

3D cell culture, bronchial mucosa, oral mucosa, colorectal mucosa, polyethylene terephthalate, poly-L-lactide.