Immobilization of Biomolecules on Chitosan Surface for Selective Recruitment, Controlled Growth and Differentiation of Cells from Mixed Populations

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One of the issues for the future of the art in Tissue Engineering is the inadequate initial interaction between polymer/surfaces and cells leading to in vivo adverse body reactions. The selectivity of biomaterial surfaces for a particular cell type present in a mixed cell population can be achieved by engineering a polymer surface with specific molecules.

In the present work, for the precise control of cell adhesion, growth and differentiation, we modified the surface of chitosan by means of covalent immobilization of different biomolecules. With this purpose, specific proteins and antibodies were activated using 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC) and the reaction as stabilized with N-hydroxysuccinimide (NHS). The reaction was optimized by manipulating multiple var- iables like time incubation, pH, protein concentration and protein: EDC:NHS ratio, in order to control the activation of the carboxyl group of the proteins and thus its covalent binding to the amino groups of the chitosan surface. Quantification of immobilized al- bumin and fibronectin was performed by colorimetric and fluores- cence methods. Fluorescence microscopy using labelled antibodies and confocal microscopy were also performed. Adhesion and via- bility of leukocytes and osteoblast like cells were assessed by MTS and DNA assays. The results revealed that the use of EDC/NHS activated the immobilization of the different molecules on chitosan surfaces. Membranes modified with adhesive and non-adhesive proteins revealed distinctive cell recruitment profiles as assessed by cell adhesion and proliferation rates.