Master assignment

Extracellular Protein Identification Cytometry (EPIC) single cell analysis

One of the main methods to investigate cellular function, including stem cell differentiation and pathological state, is the analysis of extracellular matrix (ECM) deposition. Current methods such as Western blot, mass spectroscopy, or immunostaining, either require the removal of cells and processing of the ECM prior to analysis, or only allow for the analysis of the ECM of a few hundred cells or less. In the first case, the ECM is destructively harvested and cellular heterogeneity is masked, while in the latter case too few cells are analysed to be able to draw conclusions regarding subpopulations within the cell population. Moreover, these techniques can only be employed to a limited extent to analyse ECM deposition in 3D cell culture. Thus, we are working on a novel method that allows for the quantification of specific pericellular matrix proteins of individual cells within a 3D microenvironment in an high-throughput manner.

This assignment focuses on the optimalisation of this novel analysis method. You will learn about microfluidics, single cell analysis and immunostainings. The specific focus can be chosen depending upon personal preferences.

Techniques

You will perform amongst others single cell encapsulation using microfluidics, cell culture, immunostainings, single cell analysis, fluorescent confocal microscopy and (basic) image analysis. This assignment will equipped you with a broad range of different techniques relevant for tissue engineering.

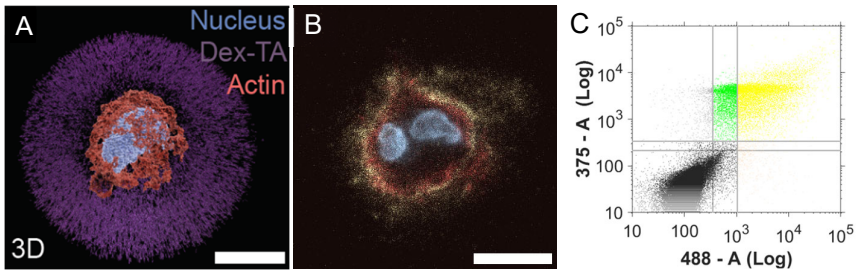

Figure 1: *A) 3D reconstruction of an encapsulated cell in Dex-TA hydrogel based on a confocal z-stack, B) Mid-section of an encapsulated cell stained for actin (red), COL I (yellow) and the nucleus (blue), C) Flow cytometry analysis results for DAPI (375) and COL I (488) stained encapsulated cells, confirming both COL I and DAPI staining (yellow population). Scale bars: 10 µm*

Supervisors

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