

Single Cell Polarization Analysis in Micro-3D Cell Culture Devices

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INTRODUCTION: The merge of micro-fabrication and cell biology allows to address so far experimentally irresolvable questions. It has been shown that the physical and chemical microenvironment of a cell plays a crucial role in controlling its function. Especially the function of epithelial cells as selective barriers between compartments demands asymmetric enrichment of lipids and proteins to specific regions of cells, which is thought to be dependent on contacts to neighboring cells. We are investigating which aspect of the polarized organization of epithelial cells can develop cell autonomously, i.e. independent on cell-cell interactions. To this end, we have developed a set of tools which enables the culturing of single cells in an array format and controlling the three dimensional shape of each individual cell.

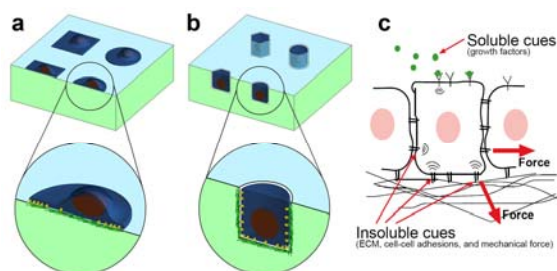


Fig.1: Scheme of the concept A) conventional 2D patterning of cells and B) micro-3D culturing of single cells. The surface of the microwells exhibits cell binding properties, while the plateau surface inhibits adsorption of proteins and attachment of cells. C) Schematic representation of cells in culture¹.

METHODS: The micro-3-D cell culturing combines 2-dimensional chemical patterning with topographical microstructuring presenting to the cells a local 3-D host structure. By the use of microfabricated Si molds and replication techniques, we have created polystyrene chips that exhibit defined microwells of various shapes. By inverted microcontact printing of a graft-copolymer, poly(L-lysine)-g-poly(ethylene glycol), which inhibits adsorption of proteins, the plateau surface between the wells could be rendered resistant to cell adhesion, while the surface inside the wells exhibited specific functions for cell attachment¹.

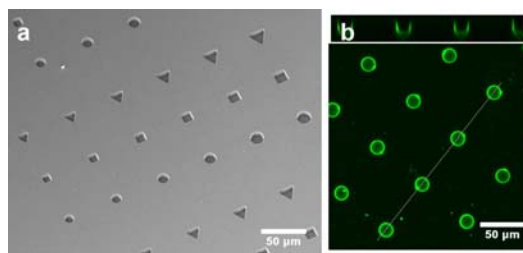


Fig. 2: Microstructured wells: a) SEM micrograph of hot-embossed structures in polystyrene b) CLSM fluorescent image after adsorption of fibronectin Alexa488 on plasma oxidized polystyrene surface, top surface stamped with a PLL-g-PEG loaded hydrogel.

RESULTS: We demonstrated that single epithelial cells can be cultured inside these microwells, remain viable and their three dimensional shape can be controlled². Furthermore, we have created arrays of microwells in other materials such as PDMS and hydrogels, which additionally allow the tailoring of the mechanical properties of the surrounding material to mimic an *in-vivo* microenvironment.

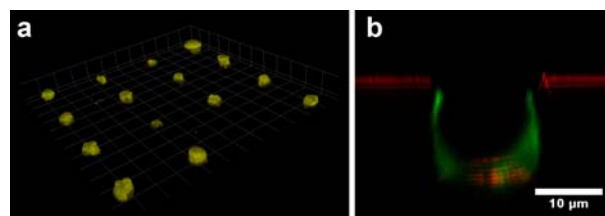


Fig. 3: CLSM 3-dimensional reconstruction of cells a) MDCK cells grown in 15 μm microwells (yellow: YFP-plasmamembrane) b) CLSM z-cross section of a single MDCK cell (transfected with GFP-actin) in a 15 μm circular well (green: actin, red: laser reflection).

DISCUSSION & CONCLUSIONS: We believe that these model surfaces are valuable tools to identify the molecular mechanisms leading to the plasma membrane polarization in epithelial cells.

REFERENCES: ¹Pirond, D.M. Chen, C. S Lab-on-a-chip for Cellomics, 2004, Kluwer Academic. 16: 1303-1313, ²Dusseiller, M. R.; Schlaepfer, D. et al Biomaterials, 2005, 26, 5917-5925.

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