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Microfabricated Cell Culture System for Single Cell Analysis in 2.5 Dimensions

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INTRODUCTION: In the last decade a number of techniques have been developed to generate microscopic patterns of biomolecules on different materials surfaces. Such chemical patterns have been successfully used as model surfaces for biorelated studies and applied to the field of biosensors, cell-surface interactions and tissue engineering. Due to the inherent complexity of cellular systems great care has to be taken to control the microenvironment of the cells[1].

It is the goal of this work to create a new type of array system to study single cells in a 2.5-dimensional microenvironment, where both topography and surface chemistry can be tailored accordingly. It is well known that the shape and adhesion state of a cell has great influence on its fate and behavior[2], but model systems to have a more complete control of the cell shape in 3-dimensions are still missing[3].

METHODS: Here we present a route to fabricate polystyrene (PS) chips with wells of 5 to 50 µm size and adjustable depth, which have been hot-embossed using a silicon rubber (PDMS) as the master. This master was replicated from a microstructured Si mold (Fig 1), produced by standard photolithography and dry etching using inductive coupled plasma (ICP). To further tailor the surface topography of the wells a simple heat treatment was used to flatten out the scalloping effect inherent in the dRIE-process. By the use of a self-assembling graft-co-polymer, poly-L-lysine *grafted* poly(ethylene glycol) (PLL-g-PEG)[4], the surface of oxygen plasma treated PS can be functionalized to be either protein repelling, thus resistant to cell adhesion, or carry a specific biofunctionality, such as cell binding peptide sequences (e.g. RGD) in any desired concentration[4]. Different routes to selectively functionalize the surface inside the wells and the surrounding plateau were investigated. Cell experiments were performed using fibroblasts (HFFs) and epithelial cells (MDCKs).

RESULTS: Some selected results are shown in the following figures. A high-throughput method to produce large quantities of structured PS films could be established (Fig 1). Preliminary results demonstrate the feasibility of creating surfaces that combine topographical features and area-selective chemical contrast (Fig 2).

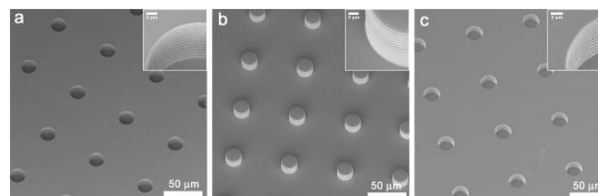

Fig 1: SEM images of a) dRIE structured Si showing an array of wells; b) replicated PDMS structures; c) hot-embossed wells in PS. Tilt angle: 30°. All structures are approximately 13 µm in depth.

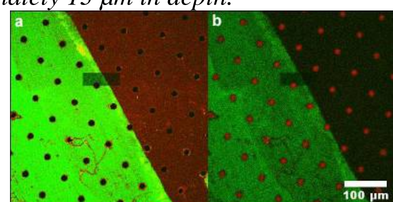

Fig 2: CLSM image of stamped border of the PS surface after µCP of PAH, adsorption of PLL-g-PEG/biotin, finbinogen488 (green channel) and streptavidin633 (red channel). a) focused on top; b) focused on the bottom of the wells.

DISCUSSION & CONCLUSIONS: The combination of cheap replication techniques, biocompatible polymers and designated surface chemistries is demonstrated to be a promising approach to produce low-cost biochips for investigating cell function and response, e.g. in the context of drug screening and development, or to create model tissues for *in-vitro* analysis. Furthermore, microstructures with optimized geometries will allow for the analysis of single cells or small clusters of cells in a controlled microenvironment, taking into account the 3-dimensional nature of cellular adhesion and mimicking more closely the *in-vivo* situation.

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