

CELL ADHESION PHENOMENA ON PATTERNED SUBSTRATES PREPARED WITH MICROCONTACT PRINTING

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INTRODUCTION: Cell adhesion, the interaction of cells with each other or with the extracellular matrix (ECM), is a complex process that plays a fundamental role during development and maintenance of the fate of multicellular organisms. The initial phase of cell/matrix interactions is characterized by the binding of specific receptors on the cell surface to ECM molecules and the assembly of the receptors at the contact sites. This leads to the induction of intracellular signalling cascades that cause the assembly of specific linker molecules at the contact sites and a reorganization of the actin cytoskeleton¹. We are interested in achieving a better understanding of the basic process of cell adhesion by confronting cells with chemically defined and regularly patterned substrates. What are the minimal requirements for initial cell adhesion? What is the maximal distance between two ECM-coated areas that can be bridged by a single cell? What is the minimum size of an ECM-coated area to induce intracellular signalling cascades, an assembly of linker molecules and a reaction of the cytoskeleton?

METHODS: We used micro contact printing (μCP)² to create well defined structures of protein-coated regions in the micro- and nanometer scale. With this method we were able to produce patterned substrates of ECM molecules (fibronectin, vitronectin, laminin) consisting of squared dots ($3\mu\text{m}$, $1\mu\text{m}$, 800nm , 500nm , 300nm) separated by nonadhesive regions of variable distances ($2\mu\text{m}$ - $30\mu\text{m}$). Cells were cultivated on the patterned substrates for 1 h, fixed and fluorescently labelled for actin, focal adhesion associated molecules (integrin, paxillin, talin, vinculin) and markers for intracellular signalling (phosphotyrosine, FAK), and analysed.

RESULTS: When cells are cultured on a homogeneous fibronectin substrate prepared with μCP , they show a typical morphology characterized by flat lamellipodia. Cells seem to interpret dots of any size as a homogeneous substrate if the distance between dots is smaller than $5\mu\text{m}$. With increasing distance between dots (10 - $25\mu\text{m}$), cells react by orienting their cell shape according to the dot pattern, forming lamellipodia with straight edges and right angles. A distance of $30\mu\text{m}$ between two dots can no longer be bridged

by a single cell, resulting in ball-shaped cells adhering to a single dot. The actin cytoskeleton reacts to dot sizes down to 500nm by forming straight bundles between dots (Fig. 1a). Integrin, focal adhesion associated molecules and markers for signal transduction accumulate in regions of the cell overlying ECM-dots (Fig. 1b) down to 300nm in side length.

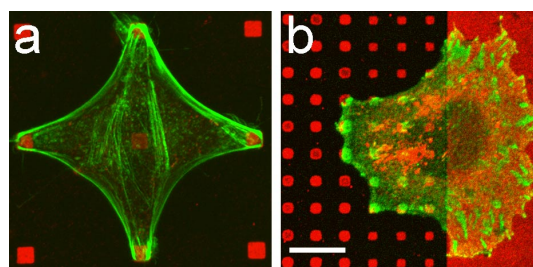


Fig. 1: (a) The actin-cytoskeleton (green) of a B16 melanoma cell on a patterned fibronectin-substrate (red). (b) BRL-fibroblast stained for phosphotyrosine (green) at the border of a homogenous and a patterned fibronectin-substrate (red). scale bar $10\mu\text{m}$.

DISCUSSION & CONCLUSIONS: μCP in combination with cell culture is a powerful technique to study basic principles of cell adhesion and migration. We show, that an ECM-coated area of $0.25\mu\text{m}^2$ is sufficient to induce a functional focal contact and that cells can bridge non-adhesive distances of $25\mu\text{m}$. We are currently using transformed cell lines expressing GFP-fluorescent proteins (integrins, tubulin, actin) to investigate the dynamics of focal adhesion formation on patterned substrates in living cells.

REFERENCES: ¹S.M. Schoenwaelder and K. Burridge (1999) *Curr. Opinion in Cell Biol.* **11**:274-286. ²M. Mrksich and G.M. Whitesides (1996) *Ann.Rev.Biophys.Biomol.Struct.* **25**:55-78

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