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CELL ADHESION ON MICRO- AND NANOPATTERNED PROTEIN-COATED SUBSTRATES: CONNECTING THE DOTS

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INTRODUCTION: Cell adhesion involves the interaction of cells with the extracellular matrix (ECM). It is mediated by integrins and is essential for the integrity and function of multicellular organisms. The initial phase of cell/matrix interaction is characterized by the binding of integrin receptors to ECM molecules and the assembly of receptors at the contact sites[1]. This leads to the induction of intracellular signalling cascades that cause the aggregation of specific molecules linking the actin cytoskeleton via integrins to the ECM[2]. Focal adhesions are therefore also the sites at which contractile forces produced inside the cell are exerted onto the substrate and, consequently, their distribution dictates the size and shape of the cell[3]. The types of ECM that cells encounter in vivo range from the homogeneous meshwork of basement membranes to the fibrillar scaffold of connective tissue or healing wounds. A cell is therefore confronted either with a nano-patterned surface or with ECM fibrils spaced in the µm range. To understand how cell behaviour is dictated by the architecture of the ECM, we exposed cells to a patterned substrate of ECM molecules obtained by the micro contact printing technique (µCP). By controlling the size of square ECM dots and the distance between them, we created nano-patterns resembling either basement membranes or connective tissue. Adherent cells were plated on these patterns, and the spreading and migration of the cells were analysed.

METHODS: We used micro contact printing[4] to create patterned substrates of ECM molecules (fibronectin, vitronectin, laminin) consisting of squared dots (3 µm, 1 µm, 800 nm, 500 nm, 300 nm) separated by non adhesive regions of variable distances (2 µm-30 µm). Cells were cultivated on the patterned substrates for 1 h, fixed and fluorescently labelled for actin, focal adhesion associated molecules and markers for intracellular signalling and analysed.

RESULTS: We first determined the capacity of cells to spread on ECM dots with increasing distance from each other. As long as the spacing of dots was less than 2 µm, cells spread as they would on a homogeneous substrate. With increasing distance between fibronectin dots (5-20 µm), cells adapted their shape to the dot pattern and grew with straight edges from dot to dot. In these cells, the actin cytoskeleton formed stress fibres between adjacent dots. When the distance between dots exceeded 25 µm, cell spreading was limited and the cells became triangular, ellipsoid or round. At 30 µm, cells adhered to one dot and did not spread.

The minimum size of an ECM dot required for cell spreading was determined using fibronectin patterns with dot sizes from 0.1 to 1 µm^2 and a constant spacing of 5 µm (centre to centre). Cells readily spread on dots which were 0.25 µm^2 or larger. In these cells, the actin cytoskeleton formed normal stress fibres connecting the dots. A dot surface of 0.1 µm^2 still allowed cell adhesion but the cells no longer spread. This indicates that cells can properly adhere to and spread on patterned ECM-coated areas equal to or larger than 0.25 µm^2 spaced at 5 µm.

We next studied the molecular composition of focal adhesions formed on patterned substrates and show by immunostaining that ß3-integrin, phosphotyrosine, focal adhesion kinase (FAK), paxillin, talin, and vinculin were localised to fibronectin- or vitronectin-coated dots, suggesting that classical focal contacts were formed. The accumulation of these molecules at the dots occurred rapidly and was already visible 10 min after plating. They even accumulated over dots of 0.1 µm^2, showing that the size of focal contacts is determined by the microenvironment and can be much smaller than previously described in the literature. Clustering of focal adhesion molecules occurred only when cells were grown on ECM molecules. When plated on patterned substrates prepared with polylysine, cells were able to adhere but actin stress fibres were not formed and accumulation of focal adhesion molecules did not occur.

DISCUSSION & CONCLUSIONS: µCP in combination with cell culture is a powerful technique to study basic principles of cell adhesion and migration. We show, that the limits for cell adhesion are a maximal distance of 25 µm between adhesive surfaces and a minimal size of 0.25 µm^2 of an adhesive dot. Knowing these limits will

allow a better understanding of in vivo cell behaviour in situations such as embryogenesis, wound healing and leukocyte migration and it will be essential for the design of implants with artificial surfaces allowing optimal interaction with cells in a tissue.

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: [1]Burridge,K. & Chrzanowska - Wodnicka,M. Focal adhesions, contractility, and signaling. *Annu Rev Cell Dev Biol* **12**, 463-518 (1996). [2]Yamada,K.M. & Geiger,B. Molecular interactions in cell adhesion complexes. *Curr Opin Cell Biol* **9**, 76-85 (1997). [3]Ingber,D.E. Tensegrity: the architectural basis of cellular mechano-transduction. *Annu Rev Physiol* **59**, 575-599 (1997). [4]Mrksich,M. & Whitesides,G.M. Using self-assembled monolayers to understand the interactions of man- made surfaces with proteins and cells. *Annu Rev Biophys Biomol Struct* **25**, 55-78 (1996).

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