

**SELF-ASSEMBLED PEPTIDE FUNCTIONALIZED HYDROGELS**S.Tugulu<sup>1</sup>, H.-A.Klok<sup>1</sup>, A.Bernd<sup>2</sup>, M.Möller<sup>3</sup>, J.Groll<sup>3</sup> & J.P.Spatz<sup>4</sup>

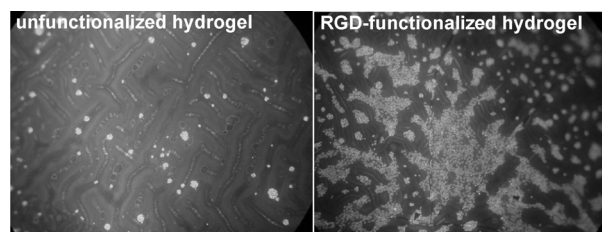
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**INTRODUCTION:** Integrins play a major role in the mechanosensory system enabling cells to sense the topology and mechanical properties of their environment as well as to detect mechanical stimuli. The main events in the function of this mechanosensory system are the binding of integrins to extracellular ligands, the clustering of integrins in the plane of the cell membrane and the aggregation of signalling molecules, linker proteins and other transmembrane receptors for extracellular signalling molecules. The resulting large protein complexes, known as focal adhesion complexes transduce the mechanical stimulus into a chemical signal, which results in an alteration of the expression of specific gene products<sup>1</sup>. We present the development of a well defined model system enabling the systematic investigation of the (combined) effects of (bio)chemical, topological and mechanical stimuli on integrin dependent cellular behaviour. The model system consists of an intrinsically bioinert hydrogel layer of cross-linked PEO-PPO starpolymers deposited on a flexible PDMS support. The functionalization of the hydrogel with short peptide ligands acting as integrin specific recognition motifs promotes adhesion of human keratinocytes and fibroblasts to its surface. The resulting substrate allows mechanical deformation of the cells in elongation experiments. Furthermore topological aspects are taken into account by elaborating several complementary approaches to control the concentration, spatial distribution and clustering of peptide ligands on the surface of the substrate on a length scale ranging from several nanometers up to a few micrometers.

**METHODS:** PDMS and silicon substrates are coated with the hydrogel layer by cross-linking isocyanate terminated PEO-PPO starpolymers<sup>2</sup>. Peptide ligands are prepared by standard Fmoc-SPPS and grafted on the substrate by using maleimide succinimide cross-linking chemistry. Peptide functionalized starpolymers are synthesized by organic chemistry procedures and characterised by standard analytical methods (RHPLC, NMR, Maldi, GPC, etc.). Surface characterisation is carried out by XPS and radiolabeling. Cell adhesion experiments are performed using human keratinocytes (HaCaT 41), which are stained with Hoechst reagent and analysed by fluorescence microscopy.

**RESULTS:** First results were obtained using a system consisting of a 25 nm thick hydrogel layer on

a silicon substrate. Peptide ligands bearing a cysteine residue were covalently bound to residual amino groups on the surface of the hydrogel via a maleimide succinimide cross-linker. A direct proof for the successful functionalization was obtained from XPS experiments by the appearance of a sulfur signal on the peptide functionalized surfaces resulting from the sulfhydryl group of the cysteine moiety. An indirect evidence was given by the clearly enhanced adhesion of HaCaT 41-cells after the peptide functionalization.



*Fig. 1: Fluorescence microscopic images of HaCaT 41 cells after Hoechst staining adhering to an unfunctionalized hydrogel (left) and to a hydrogel functionalized with the peptide sequence GRGDSC (right).*

**DISCUSSION & CONCLUSIONS:** We were able to show, that integrin specific cell adhesion can be accomplished on an otherwise bioinert hydrogel by the functionalization with peptide ligands. Future work will include extending the functionalization strategy to elastic substrates and the introduction of topological variations in the presentation of the peptide ligands on the surface.

**REFERENCES:** <sup>1</sup>C.K. Miranti, J.S. Brugge (2002) *Nature Cell Biol* 4:83-90 <sup>2</sup>H. Goetz, U. Beginn, C.F. Bartelink, H.J.M. Grünbauer, M. Möller (2002) *Macromol. Mater. Eng.* 287:223-230.

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