

# MICROPATTERNS OF FUNCTIONAL PROTEINS FOR CELL CULTURES

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**INTRODUCTION:** The importance of topological and chemical surface structures on the behaviour and the functionality of cultured cells is well recognised. Various cell types, however, react differently and little is known about the *specific* reactions of most cell types. For a more detailed investigation on the interaction of cells with structured surfaces micropatterns of specific proteins have been developed.

Adhesion proteins are especially interesting for cell-surface investigations. Gene constructs of the chicken neuron membrane proteins axonin-1 and NgCAM have been generated wherein the transmembrane part has been replaced by a sequence encoding for a peptide-linker ending with a C-terminal cysteine [1]. The thiol-group of Cys permits a stable immobilisation of the recombinant adhesion proteins directly on gold surfaces or alternatively on other materials, which have previously been functionalised with maleinimid-groups.

**METHODS:** In order to achieve a stable immobilisation, glass surfaces were first silanised and by using heterobifunctional crosslinkers the recombinant proteins were covalently attached. Standard positive photoresist techniques were adapted to generate micropatterns of proteins on glass. Both lift-off and plasma etching techniques were used to transfer the photoresist pattern into a layer of the covalently immobilised peptides or protein. Fragile proteins such as the mentioned adhesion proteins have to be protected by a thin sucrose layer in a first step. By a combination of the lift-off and the etching technique complementary patterns of two different proteins were generated.

**RESULTS:** Neurons were cultured on the unstructured chips and we observed six times longer neural outgrowths on NgCAM than on aminosilane, whereas on axonin-1 the mean lengths increased only by a factor of two [2]. This result shows that the adherence of cells strongly depends on the surface chemistry and that immobilised adhesion proteins specifically induce cellular reactions. We didn't observe a decrease in the biological activity of the immobilised proteins during several weeks.

By using the microstructuring techniques grids of axonin-1 were produced on glass chips. In cultures of dissociated neurons from chicken dorsal root ganglia the cells preferentially adsorb to the knots

in the grid. Their neural outgrowths spontaneously align along the protein lines. Thus, micropatterns of specific adhesion proteins are useful to establish neuronal networks. Furthermore, arrays of gold microelectrodes have been produced, which allow us to address individual cells. The preliminary electrophysiological measurements look promising. When specific adhesion proteins are present on electrodes the neuron-electrode contact may be improved as measurements of the mean distance of the cell membrane to the material surface indicate [1].

When micropatterns of two different proteins are generated on one surface, a resolution of 2  $\mu\text{m}$  could be achieved. The functionality of the two proteins streptavidin and IgG has been tested using fluorescence microscopy: the areas where the added biotin-fluorescein conjugate was bound appeared in green, the IgG areas with the bound second-antibody-rhodamine-conjugate were red.

**DISCUSSION & CONCLUSIONS:** The techniques described here have a high potential for more general applications in biomaterials research. The generation of specific protein patterns allows us to establish co-cultures of two different cell types. Recently, it became evident that glia cells play a more active role in synaptogenesis than previously assumed [3]. It can be assumed that a close contact of two different cell types is also important for many further complex biological processes. Thus, co-cultures on predefined microstructured patterns may play a key role in the future research of biomaterials.

**REFERENCES:** <sup>1</sup> H. Sorribas, D. Braun, L. Leder, P. Sonderegger and L. Tiefenauer (2001) *J. Neurosci. Meth.* **104**:133-141. <sup>2</sup> H. Sorribas, C. Padeste, T. Mezzacasa and L. Tiefenauer (1999) *J. Mater. Sci.: Mater. Med.* **10**:787-791. <sup>3</sup> E. Ullian, S. Sapperstein, L. Christopherson and B. Barres (2001) *Science* **291**:657-660.

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