

NEUROCHIPS FUNCTIONALIZED WITH ADHESION PROTEINSH. Sorribas¹, C. Padeste¹, P. Sonderegger², C. Stricker³ [L. Tiefenauer](#)¹¹[Paul Scherrer Institut](#), Villigen, Switzerland²Institute of Biochemistry University of Zürich, Switzerland³Institute for Neuroinformatics, Univ. & ETH Zürich, Switzerland

INTRODUCTION: Various types of microelectrode arrays (MEA) have been produced using photolithographic techniques. Such MEAs are used for stimulating and recording neural activity in slices of nerve tissues or of dissociated cultured neurons. In order to guide neurites of neurons to gold electrodes and also to achieve a close cell-electrode contact, we have generated patterns of novel neural adhesion proteins.

METHODS: Recombinant proteins from the two membrane proteins axonin-1 and NgCAM have been produced using protein engineering methods. These recombinant proteins have a C-terminal Cys, which allows the direct coupling to gold surfaces; alternatively a covalent immobilization on glass or oxides can be achieved using a silane and a heterobifunctional crosslinker. The cell-surface distance was determined using the fluorescence interference method [1]. Furthermore, photolithographic methods were applied to generate patterns of functional proteins [2]. The quality of these biopatterns was assessed by immunofluorescence microscopy of adhered neurons. Microelectrode arrays have been fabricated according to [3] and biofunctionalized with RGDC. These chips have been tested in first electrophysiological experiments.

RESULTS: On glass a line pattern of covalently immobilized axonin-1 was generated. Dissociated neurons spontaneously adhere to these lines (Fig. 1). The resulting network is not yet perfectly aligned and cell adhesion to the background may additionally be suppressed.

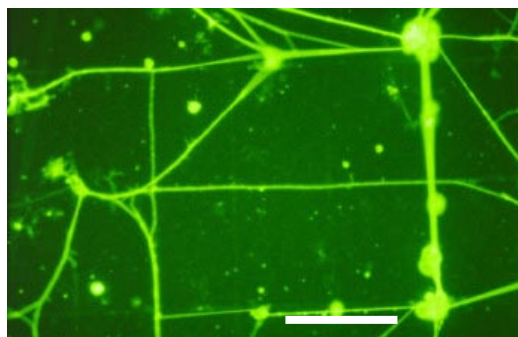


Fig.1: Primary neurons cultured on patterned Cys-axonin patterns (5 μm line width, immunostained with TRITC anti-axonin-1, scale bar 100 μm).

In Table 1 mean lengths of outgrowing neurites and mean distances of the neuron cell membrane to the surface for different modifications are summarized. It is evident that the adhesion proteins axonin-1 and NgCAM promote neurite outgrowth and that the cell membrane is in a minimum distance to the material surface with these proteins. In addition it was found that on NgCAM and laminin 80% to 90% of all cells have outgrowths whereas only 20 % of the neurons form neurites on aminosilane (APTES). This result confirms the assumption that the adhesion protein strongly influences the biological reaction of cultured cells. Thus, depending on the intended application another adhesion molecule can be selected.

Table 1. Neuron cultured on various substrates

Substrate	Mean neurite length [μm]	Cell-surface distance [nm]
Cys-axonin	80 \pm 30	37 \pm 10
Cys-NgCAM	231 \pm 86	47 \pm 8
APTES	40 \pm 10	39 \pm 3
RGDC	124 \pm 60	39 \pm 4
laminin	n.d.	91 \pm 4

Finally, the neurochips were tested in an electrophysiological experiment.

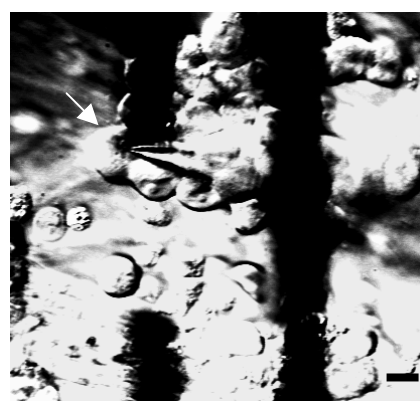


Fig.2: Differential interference contrast micrograph of a dissociated DRG culture (4d) with a gold electrode (black rectangular structure) for stimulation and impaled patch pipette for recording (scale bar 10 μm).

A microelectrode close to a neuron cell (Fig.2, white arrow) has been addressed and a current of

150 μ A has been applied for 100 μ s. This stimulation induced the expected action potential of about 90 mV (see Fig. 3) as measured with a patch clamp electrode. This preliminary result confirms that our microelectrodes are useful for electrophysiological experiments.

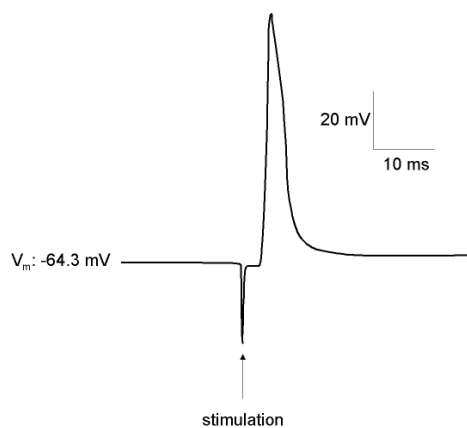


Fig.3: Action potential recorded intracellularly after extracellular stimulation.

DISCUSSION & CONCLUSIONS: The results demonstrate the potential of specific adhesion proteins immobilized in patterns on neurochips. Lines of adhesion molecules induce the adherence of cells to predefined areas and guide their outgrowths. Furthermore, some adhesion proteins can also provoke specific biological reactions. For instance, the induction of synapses at predefined location, preferentially on electrodes, is most interesting.

The techniques developed for neurochip production have also a potential for many other applications. Especially, micropatterns of functional proteins of interest can be generated and the functionality can spatially be controlled with a resolution of 1 μ m. The described photolithographic method allows us also to produce many chips on one wafer and is thus suitable for a mass production. Functional micropatterns may be required for multiplex sensing. The mentioned method can also be adapted to produce pixel gradients out of two different molecules. Such biosurfaces may be very interesting in order to investigate and control the migration of cells.

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ACKNOWLEDGEMENTS: This work was supported by the SNF SPP Biotechnology, Module Neuroinformatics. We thank L. Leder for the preparation of the adhesion proteins axonin-1 and NgCAM.