European Cells and Materials Vol. 6. Suppl. 1, 2003 (page 48) ISSN 1473-2262
Poster ID: 126

MICROPATTERNED NEURAL NETS ON DIFFERENT SOLID SURFACES

M.Jungblut, C.Schwind, W.Knoll, K.Graf & C.Thielemann

Max Planck Institute for Polymer Research, Mainz, Germany.

INTRODUCTION: Precise control over the surface composition of materials allows either the reduction or the promotion of cell growth. Patterning of the surface properties is achieved by microcontact printing (μCP) of adhesion proteins with a PDMS (polydimethylsiloxane) stamp. This enables the structured adhesion of primary cortical neurons by allowing the cells to be placed on well-defined areas of the substrate or device. Our motivation for biopatterning surfaces with glass and gold substrates is the controlled growth of neural nets on a planar microelectrode array (MEA) chip. Coupling between electronic devices and neurons allows for extracellular signal recordings.

For neuropatterning on three-dimensional MEA structures we employed a new, easy-to-handle method based on the local swelling of pre-structured polymer substrates [1]. This method allows us to prepare scalable arrays of regularly shaped microvessels that contain chemically modified gold electrodes at the bottom, representing the adhesive areas for the neural cell bodies.

METHODS: The μCP technique was used to adsorb poly-D-lysine (PDL) in a grid pattern to glass surfaces, thin gold films and to MEAs. We used two types of MEAs. One of which is commercially available from MultiChannel Systems (Reutlingen, Germany); the other of which we designed. Prior to stamping, the gold surfaces were treated with mercaptoundecanoic acid (MUA), an alkanethiol that forms a self-assembled monolayer, allowing the tight binding of a PDL pattern.

The PDMS stamps were soaked in a solution of sterile poly-D-lysine (0,1 mg/ml in PBS) and brought in contact with the surface for 10 minutes at room temperature. We used and optimized grid pattern geometry [2] with a line width of 5 μm an a line distance of 50x100 μm; ideally the cross points would form the adhesion points for the cell bodies, while the lines would guide the outgrowth of the neurites. For the MEAs, the PDL pattern is optically adjusted to the electrode geometry.

The three-dimensional arrays of microvessels with a 50 μm^2-square-range to area contain gold at the bottom of microwells to change their hydrophilicity upon adsorption of thiols containing hydrophilic end-groups. The array was soaked in a solution of laminin (25 μg/ml PBS) which subsequently adsorb to the hydrophilic microvessels.

The cortical cells were obtained from 19-day-old rat fetuses (E19) and plated onto the surface in densities between 100 and 300 cells/mm^2 and cultured at 37 °C an 5% CO_2 in serum free B27/Neurobasal medium with 0,5 mM glutamine.

RESULTS: Cortical cells were found to grow in patterns on glass and the thiol-treated gold surfaces after the PDL pattern transfer by μCP. The surface of the self-fabricated MEAs consisted of silicon dioxide and gold electrodes and so it was possible to form a pattern of neurons on the array. We were also successful in patterning the cortical cells on a commercial microelectrode array. The cells on the arrays of microvessels were growing inside the microwells and form connections to other cells. Furthermore, we proved that patterned neurons formed synapses, which is the condition for interneural communication.

DISCUSSION & CONCLUSIONS: We patterned cortical neurons by μCP on a variety of substrates. The conditions for the formation of a vital, synaptically connected network are an optimal pattern geometry and the right “ink” to form structures that promote cell-growth. The transfer of this neuropatterning technique to the surface of a microelectrode array chip will allow us to record extracellular signals of individual cells in the network. These signals are of fundamental interest for basic neuroscience studies, cell-based biosensor technology and tissue engineering. Neuropatterning on three-dimensional microvessel arrays allows us to substitute the μCP by a self-adjusting cell adhesion process but also offer the cells a compositionally attractive surface. These devices can be modified in order to realize three-dimensional MEA chips. Future work will focus on the recording of the whole network response towards input stimuli patterns.

REFERENCES: [1]E. Bonaccurso, H.-J. Butt and K. Graf, Microarrays by structured substrate swelling, subm. to EPJ. [2]Lauer L., Klein C., Offenhäusser A., Spot compliant neuronal networks by structure optimized micro-contact printing. Biomaterials 2001;22:1925-32