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SURFACE STRUCTURING METHOD FOR DEFINED GROWTH OF NEURAL NETWORKS

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INTRODUCTION: Culture studies in vitro provide better control over the extracellular milieu, thereby allowing one to change systematically the environmental conditions around cells. Surface patterns in high resolution have been produced down to the micrometer scale, e.g. by using standard photolithography [1], combination of laser ablation techniques with lithographic masks [2] and microcontact printing [3]. Most of these methods are based on precise templates, which are used as aperture stops, punching tools or they are engraved onto the cell substrate itself. A method is presented to fabricate cell patterns. By designing patterns with a computer controlled laser ablation process the desired structures can be realized within few minutes without the need of a precasted template.

METHODS: Glass slides were silanized with OTS (octadecyltrichlorosilane) and a silicone chamber was fixed on it. Then the hydrophobic surface within the chamber was coated with laminin which is a protein of the extracellular matrix. A pattern was burned in the laminin coated layer by a computer controlled UV-laser ablation. The pheochromocytoma cells (PC12) were positioned on nodes of the pattern by the use of optical tweezers. Nerve growth factor (NGF) was added to induce neurite growth and to inhibit repeated cell divisions.

RESULTS: We formed an array of three times three octagons on the laminin-molecules coated glass slides with a node size of about 10 to 30 µm and a line thickness of 2 to 10 micrometers, whereby the interior of the octagons was ablated with the help of an UV-laser.

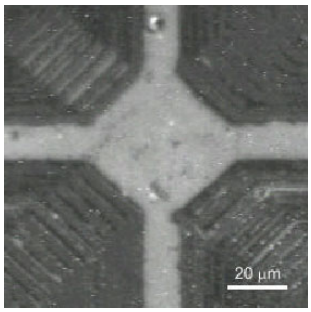

Fig. 1: Fluorescence image of a laminin coated, node between ablated areas which were incubated with Cy3.5-labeled anti-laminin at RT.

The fluorescence image (Fig 1.) showed, that the ablation process was effective and that the laminin molecules were removed or destroyed. After 3-4 days the PC12 cells formed a small network according the burned pattern, which was only affected by some cell divisions (Fig 2.). The cells and their neurites don't grow on the ablated areas.

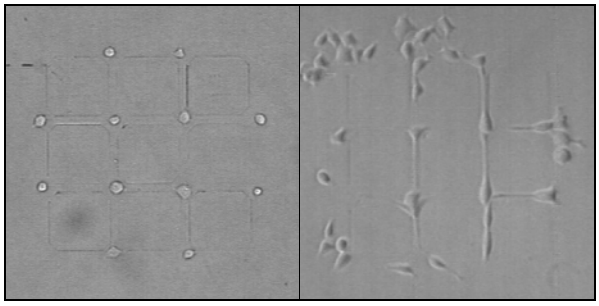

Fig. 2: PC12 cells on laminin coated glass slides

DISCUSSION & CONCLUSIONS: This method is very versatile and can be performed in absolute sterility. No substantial preparatory work like the execution of complex surface chemistry or mask fabrication is necessary. After the unspecific absorption of laminin molecules on the OTS-coated cover glasses, a computer-controlled ablation process is started which follows a programmed pattern and is in our case an arrangement of octagons with laminin-coated lines and nodes between them. It is possible to keep the time between desired design and realization of the pattern short especially in comparison to other methods. This is an enormous advantage if one wants to test cell growth on many patterns which by comparison takes a much longer time with other methods and is considerably extensive.

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