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Neuronal adhesion and growth on polyethylenimine tracks microprinted on polyethylenoxide-polypropylenoxide coated surfaces

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Abstract-**Adsorbed layers of polyethylenoxide-polypropylenoxide (PEO-PPO) blockcopolymers were tested as neurophobic background coatings in neuronal patterning studies over a time period of 30 days. Microprinted tracks of polyethylenimine (PEI) were used as the neuron-adhesive part of the pattern. Results showed that PEO-PPO surfactants F108 and F127 (Synperonics, ICI) significantly reduced the growth of neuronal tissue when adsorbed on Polyimide and Fluorocarbon surfaces up till 8 days. Also survival of neuronal tissue was not negatively affected by the PEO-PPO. Viability of the cultures was assessed after 30 days and showed viability factors above 0.9 (scale 0 to 1).**

Key words **- Cortical neurons, patterning, adhesion, polyethylenimine (PEI), PEO-PPO, viability**

I INTRODUCTION

One of the key problems in long-term neuronal patterning studies on multi-electrode arrays (MEAs) is the biofouling of the background material with cell-adhesive proteins, which in turn promote the random overgrowth with neuronal tissue [1]. Polyethylenoxide (PEO)-coated surfaces are known for their ability to inhibit the adsorption of proteins and are promising alternatives as neurophobic background surfaces. The methods to fabricate PEO-coated surfaces can be divided in two different subgroups e.g. covalent bonding of relatively short polyethylenoxide chains (PEO also termed polyethylenglycol PEG) and adsorption of polyethylenoxide-polypropylenoxide (PEO-PPO) blockcopolymers onto hydrophobic materials [2]. The advantage of covalent coupling of PEO chains to surfaces is the initial stability of the layer and the prohibited displacement of PEO by proteins in solution. However, the chemical bonds between the PEO chains and the underlying substratum could be dissociated by the physiological surrounding in time. Another important point is the fact that the chemistry involved should be transferable onto multi-electrode arrays, which are usually vulnerable to more laborious chemistry. For instance, cleaning of MEAs with aggressive acids before chemical modification is prohibited because the conductive metal leads would be dissolved. Therefore, it is relevant to test more simple modification routes as potential methods to be used on MEAs. Thus, adsorbed layers of PEO-PPO blockcopolymers on hydrophobic surfaces were investigated as potential neurophobic background surfaces in patterning studies.

The aim of this paper is to study the adhesion, growth, and survival of cortical neurons on polyethylenimine (PEI) patterns microprinted on PEO-PPO-treated surfaces. The efficacy of the adsorbed blockcopolymers as neurophobic background surfaces was evaluated over a time period of 30 days.

II. MATERIALS AND METHODS

A. Microprinting PEI onto PEO-PPO backgrounds:

The triblock copolymers F108 (EO_{127}-PO_{48}-EO_{127}; ICI) and F127 (EO_{95}-PO_{62}-EO_{95}; ICI) were dissolved in 0.1 M phosphate buffered saline (1% w/w) and adsorbed (24 hours) onto Polyimide (PI, Probimide 7510®, Arch Chemicals N.V., Zwijndrecht, Belgium) and Fluorocarbon (FC)-coated samples. Samples were rinsed with water (Aqua Purificata, Bufa BV, Uitgeest, The Netherlands) and dried by vacuum aspiration.

Polydimethylsiloxane (PDMS) stamps were heat cured (120°C, 25 min.) in molds (gold) and made hydrophilic by short exposure to the flame of a gas burner. Stamps were wetted by pressing the stamp (15s.) into dust-free clean room tissue soaked with PEI (10 mg/ml). To avoid the danger of capillary effects, evaporation of the residual water was visually checked until no changes were observed anymore. Finally, stamps were pressed onto background materials using a pressure load of 0.55 kg/cm^2 (15 s.).

B. Cortical neuron isolation and procedures:

Dissociated (Trypsin/EDTA) cortical neurons (neonatal rat cerebellum) were seeded onto patterned structures with a plating density of 5000 living cells/mm^2. Cells were allowed to adhere onto the surfaces during a time period of 4 hours. Samples were rinsed with NaCl (0.9%) solution to remove non-adherent cells. Neurons were cultured in R12 medium (DMEM/HAM's F12, Gibco) without serum.

C. Quantification of background adhesion and viability:

Digital microphotographs (AxioCam HR, Carl Zeiss, Germany) were taken on 2 separate subsections of each pattern after 1, 4, 8, 15 and 30 days. Each time laps procedure was done on 3 different samples. Bitmap images were stored with 1030-1300 pixel resolution prior to image processing in Matlab (Version R11.1, The Mathworks Inc). Quantification of the combined effect of neuronal adhesion and overgrowth on the background materials was assessed by a tresholding procedure taking into account disturbing non-uniform illumination effects that are usually present in images. To assure the fact that only adhesion and overgrowth on the back-

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ground materials was investigated, tissue on the PEI tracks were disregarded in the analysis.

Viable and non-viable tissue was evaluated with a staining procedure using acridine orange (AO) and propidium iodide (PPI) respectively. A viability factor V was defined as the ratio of the area covered with viable neurons A_V with respect to the summation of areas covered with viable tissue A_V and non-viable tissue A_{NV}

$$V = \frac{A_V}{A_V + A_{NV}} \qquad (1)$$

III. RESULTS

The adsorbed PEO-PPO layers F108 and F127 both suppressed the adhesion and growth of neuronal tissue onto Polyimide (water contact angle PI = 73 degrees) and Fluorocarbon (water contact angle FC = 95 degrees) surfaces. After 1 days the effect was most significant on Polyimide. After 8 days, the difference is still significant except for the F127-PI combination. After 30 days, differences are not significant anymore. The time-stability of microprinted tracks of PEI could be checked by microscopic inspection of samples before and after culture periods of 30 days.

The viability V was calculated after 30 days and showed no significant differences between cultures tested. Viability factors above 0.9 (scale 0 to 1) were calculated for all cultures.

IV. DISCUSSION

F108 and F127 PEO-PPO blockcopolymers are known for their ability to form "brush-like" surfaces with protein repellent PEO chains sticking out into the aqueous environment and PPO blocks anchoring onto the hydrophobic background [3]. The most profound neurophobic effect of PEO-PPO was therefore expected on the more hydrophobic FC. Surprisingly the results seem to be slightly in favor of the F108-PI and F127-PI surfaces. Surface parameters like roughness on the nanoscale probably influence the conformation of the PEO-brushes formed.

Neurotoxic effects can be excluded as viability was comparable on cultures tested with and without F108 and F127. Displacement of adsorbed F108 and F127 blockcopolymers by adhesive proteins (Vroman effect) is not likely to occur within 1 day after seeding because neuronal tissue was inhibited on the background surfaces.

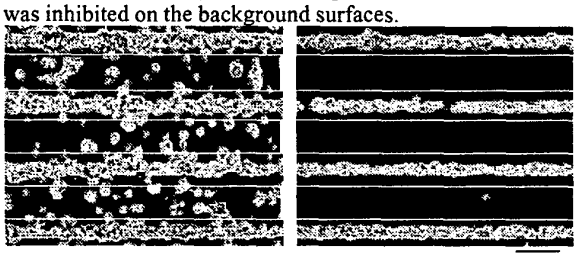

Fig.1. Matlab processed image (1 day) of adhering neuronal tissue on PEI microprinted patterns. Background material is Polyimide (Left) and F127 adsorbed on Polyimide (Right). Scaling bar = 100 µm. Analyzed background areas were located between the white lines.

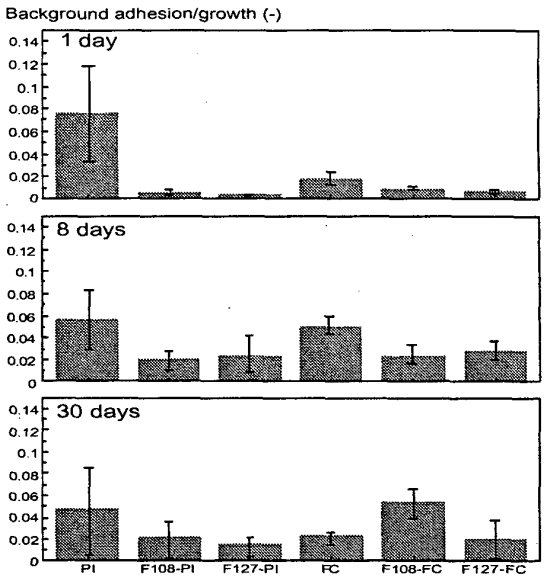

Fig. 2. Comparison of combined adhesion and growth of neuronal tissue on different background surfaces over a time period of 30 days. The mean ± SD (6 images) collected from 2 tissue isolation experiments, is presented.

V. CONCLUSION

PEO-PPO surfactants F108 and F127 (Synperonics, ICI) significantly reduced the growth of neuronal tissue when adsorbed on Polyimide and Fluorocarbon surfaces up till 8 days. In addition, survival of tissue was not negatively affected by the F108 and F127. The simplicity of the method offers a practical alternative to improve the neurophobicity of hydrophobic background materials (insulation layers) of multi-electrode arrays.

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