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Adhesion and patterning of cortical neurons on polyethylenimine and fluorocarbon-coated surfaces

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Abstract – **In this study adhesion and patterning of cortical neurons on modified glass surfaces was investigated. Patterns of cortical neurons were prepared with a combination of polyethylenimine (PEI) and plasma-deposited fluorocarbon (FC). In addition neurite development and fasciculation of interconnecting neurites between PEI-coated areas was studied. The patterns consisted of PEI-coated circular holes (diameter 150 μm) which were initially etched in a Fluorocarbon (FC) layer. The separation distance between the PEI-coated circular holes was varied from 10 up to 90 μm. This paper shows that the chemical patterns, prepared with a combination of polyethylenimine (PEI) and plasma deposited Fluorocarbon (FC), results in highly compliant patterns of adhering cortical neurons. Furthermore it was shown that interconnecting neurite bundles between neurons on the PEI-coated circular holes were especially present on the pattern with a minimal separation distance (10 μm) between the PEI-coated circular holes. In contrast interconnecting neurite bundles were hardly observed on patterns with a maximal separation distance (90 μm) between the PEI-coated circular holes.**

Key words – **Cortical neurons, Polyethylenimine, Fluorocarbon**

I. INTRODUCTION

Maintainance of patterned neurons on planar surfaces is most likely to be achieved with a stable and chemically inert combination of adhesive and non-adhesive materials. Coatings of biomolecules [1] or frequently used chemical coatings [2,3] do not fully meet these necessary requirements to survive under physiological circumstances over longer periods of time.

Many authors stressed the important role of Fluorocarbon (FC) coatings as tools to prepare non-adhesive surfaces for cells [4,5]. The properties of FC-coatings resemble the properties of Polytetrafluoroethylene (PTFE), a material with exceptional thermal, electrical and chemical properties [6]. The strong irreversible adsorption of albumin on hydrophobic PTFE is considered to be the important factor in preventing specific binding between cells and PTFE [1,6]. The properties of PTFE are considered to be indicative for a whole range of FC-coatings. Some techniques that are available to deposit FC-coatings on surfaces are spin-coatings of commercially available products, plasma deposited coatings of carbonhydro-trifluoride (CHF_3) and E-beam evaporated coatings of PTFE [7].

Cell adhesive coatings with the ability to maintain their surface properties in a physiological environment are rare. Examples of disturbing processes are the desorption of the top layer of self assembled monolayers of thiols on gold surfaces [8] or the hydrolysis of silanes [9] and polypeptides in other studies. The application of these coatings is therefore more questionable for long-term patterning of cells. However the choice for many of these coatings is driven by the supposed electrostatic interaction between positively charged aminogroups and negatively charged phospholipids in the cell membrane [10]. A clear example is poly-L-lysine with a high density of positively charged amino groups in the peptide chain [11]. An interesting alternative and relatively unknown material in the field of neuroscience is polyethylenimine [12]. Polyethylenimine (PEI) in aqueous solution contains a relatively high density of positively charged aminogroups in the polymer structure and is expected to be more bioresistant due to the lack of amide linkages in the polymer backbone. PEI behaves like a positively charged polyelectrolyte in an aqueous solution below pH 9 and fixates electrostatically onto negatively charged materials like mica or silicondioxide [10,12]. The unique electrostatic properties of PEI offer the possibility to form a stable binding between neuron membranes and inert negatively charged surfaces [10,12].

The aim of this paper is to study the adhesion and patterning of cortical neurons on

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surfaces coated with a combination of plasma deposited fluorocarbon (FC) and polyethylenimine (PEI). A subgoal is the investigation of neurite development and fasciculation of neurites between PEI-coated areas embedded in the plasma deposited FC-coating.

II. MATERIALS AND METHODS

Glass (N.V.Glaverbel, Mol, Belgium) was used as a base material onto which different coatings were prepared. Initially glass samples were spin coated with polyimide (Olin microelectronic materials) which was diluted in n-methyl pyrolidon (1:1 volume ratio). Samples were post-baked at 120°C during 5 minutes to remove residual solvent. The polyimide (PI) samples were used for the deposition of neurophilic and neurophobic coatings.

Neurophilic coating and deposition

Polyethyleneimine (PEI):
A stock solution (10 mg/ml) of Polyethylenimine in water (Fluka chemie, MW between $6*10^5$ and $1*10^6$) was diluted 1000 times to reach a final concentration of 10 µg/ml. Under sterile conditions each sample was immersed into this solution during a time span of 60 minutes. Each sample was rinsed extensively in sterile millipore water for 60 seconds to remove some non-adsorbed PEI.

Neurophobic coating and deposition

Plasma deposited Fluorocarbon (FC):
Samples were coated with a FC-layer using a CHF_3 plasma (25 sccm, 150 mTorr, 10 W) for 9.5 minutes and a subsequent treatment with CHF_3 (5 W) for 1.5 min.

Fabrication procedures of patterned surfaces

In a first step the FC-layer is deposited onto the Polyimide coated glass. In a second step a pattern with circular holes (diameter of 150 μm) was etched in the combined polyimide/fluorocarbon layer using microlithography [3]. The pattern consisted of 9 different subsections with each subsection having a different separation distance between the holes (see Figure 1). The distance between the circular PEI-coated holes was varied between 10 and 90 µm. Selective removal of the combined polyimide/ fluorocarbon layer was achieved with a CHF_3/O_2 plasma (25 sccm CHF_3, 5 sccm O_2, 10 mTorr and 75 W). Subsequently the samples were immersed in the Polyethylenimine (PEI) solution for 60 minutes. Finally the protective photoresist on top of the fluorocarbon layer was lifted off with a NaOH solution (1.0 M) and simultaneously lifted off PEI from the areas around the holes.

Cortical neuron isolation

Cerebral cortex from 1 day old newborn rats was dissected out under sterile circumstances. The tissue was trypsinized (0.25 % Trypsin/EDTA) for 45 minutes in an incubator at 37 °C at 5 % CO_2 and subsequently treated with Soybean Trypsin Inhibitor (STI, 1mg/ml) and Desoxyribonuclease I (DNAse I, 1.1 unit/ml). Dissociated tissue was spun down at 1200 rpm during 5 minutes and resuspended in R12 medium. Sedimentation of cortical neurons onto patterned surfaces took place from a glass cylinder with a sedimentation depth of 5 mm. The applied seeding density of cortical neurons was $0.15*10^6$ cells/ml. After 4 hours of adhesion, samples were rinsed with a physiological salt solution (0.9 % NaCl) to remove non-adherent neurons.

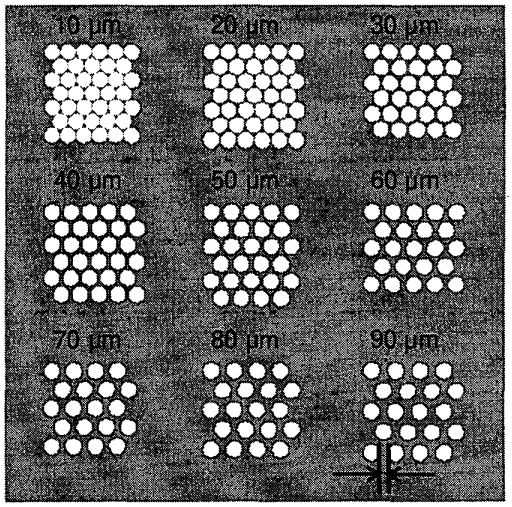

Figure 1. Overview of 9 patterns of circular holes which were used for the experiments. Dark areas represent the Fluorocarbon-coated part of surface. Light areas represent the Polyethylenimine (PEI) coated part of the surface. The numbers refer to the separation distance between the PEI-coated circular holes. Diameter of circular hole is 150 µm.

III. RESULTS AND DISCUSSION

The adhesion of cortical neurons on the patterned surfaces was investigated. Two examples of cortical

neuron adhesion after 1 day in vitro are presented in figure 2. The results emphasize some typical phenomena observed during the experiments. Polyethylenimine (PEI) was an excellent neurophilic coating and supported the adhesion of cortical neurons inside the PEI-coated circular holes to a significant extent. Statistical analysis revealed that 85 ± 10 % of the total number of observed neurons were located inside the PEI-coated holes on patterned surfaces with only 39 % covered with PEI-coated circular holes (see figure 2, right side). The CHF_3 plasma deposited Fluorocarbon layer served as a non-adhesive coating for cortical neurons (see figure 2).

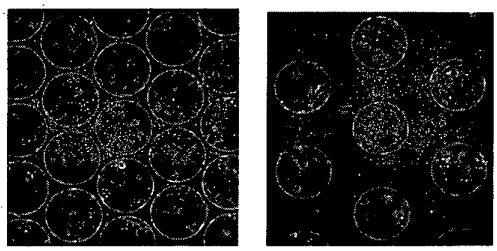

Figure 2. Phase contrast images of cortical neuron distribution on patterned surfaces after 1 day in vitro. Left: cortical neuron distribution on a pattern with a separation distance of 10 μm between the PEI-coated circular holes. Right: cortical neuron distribution on a pattern with a separation distance of 90 μm between the PEI-coated circular holes.

Figure 3 shows morphological features of the developing cortical neurons after 8 days in vitro on the same sections as shown in figure 2. A clear clustering of neurons into aggregates is visible on both patterns. The centre of the neuron aggregates is frequently positioned inside the circular hole when the holes are wide apart from each other (see right side of figure 3). On the pattern with small distances between the circular neurophilic holes, aggregates can also be found in between. The process of neurite formation and subsequent development of interconnecting neurite bundles between circular PEI-coated holes is shown in figure 3. Figure 4 shows the corresponding quantitative results for the 9 different patterns. Due to the structure of the 9 patterns, each PEI-coated circular hole is surrounded by 6 other PEI-coated circular holes. Thus the number of interconnecting neurite bundles between a neurophilic hole and its surrounding holes is always limited to a maximum number of 6. The results in figure 4 demonstrate

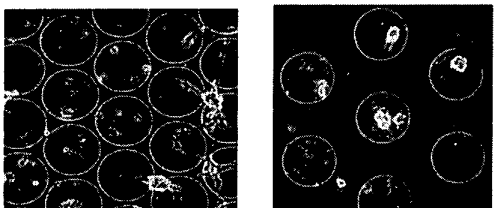

Figure 3. Examples of cortical neuron aggregation and development of interconnecting neurite fibers between PEI-coated holes after 8 days in vitro on patterned surfaces with typical separation distances of 10 μm (left) and 90 um (right) between the PEI-coated circular holes.

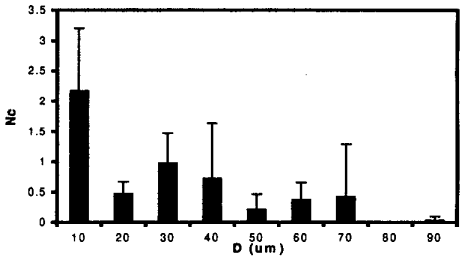

Figure 4. Average number Nc of observed interconnecting neurite bundles between a PEI-coated circular hole and the 6 surrounding PEI-coated holes vs. the separation distance D between the PEI-coated holes. Mean ± S.D. (calculated over 5 images).

that interconnecting neurite bundles between neurophilic PEI-coated circular holes are especially present on the pattern with a minimal distance of 10 μm between the neurophilic PEI-coated circular holes (see figure 3, left side). In this case, the average number of connections still was only 2.2 and did not approach the maximal number of 6. The conclusion is that the CHF_3 plasma deposited fluorocarbon layer is a non-adhesive surface for cortical neurons and additionally serves as a barrier for developing neurites. Almost complete isolation of neurons and neurites into neurophilic PEI-coated holes was obtained with a separation distance of 90 μm between the neurophilic PEI-coated holes.

IV. CONCLUSION

The plasma-deposited fluorocarbon layer is a non-adhesive coating for cortical neurons and can be designated as neurophobic. Polyethylenimine (PEI) supports neuronal adhesion and can be designated as neurophilic. Isolation of cortical neurites and fasciculated neurites into neurophilic PEI-coated

circular holes is maximal if the distance between the neurophilic areas is at a maximum.

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