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Electrically controlling cell adhesion, growth and migration

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ABSTRACT

We have developed a neurochip to control the adhesion and outgrowth of individual neurons by electrochemical removal of protein repellent molecules from transparent electrodes. The neurochip architecture is based on three parallel indium-tin-oxide (ITO) electrodes on a SiO_2 substrate and a photoresist structure forming a landing spot for the neuron soma and two lateral outgrowth pathways for the neurites. The whole surface was turned protein and cell repellent with poly(ethylene glycol) grafted-poly(L-lysine) (PLL-*g*-PEG) before enabling neuron soma adhesion by selective PLL-*g*-PEG removal. After the neuron has settled down a potential was applied to the pathway electrodes to permit the neurite outgrowth along pathways formed by the SU8 structure. We also show the possibility to control cell migration by small pulsed currents. Myoblasts were therefore seeded on a chemical pattern of cell adhesive PLL and cell resistant PLL-*g*-PEG. The PLL-*g*-PEG was then removed electrochemically from the electrodes to permit migration onto the cell free electrodes. Electrodes without applied current were confluently overgrown within 24 h but a small pulsed current was able to inhibit cell growth on the bare ITO electrode for more than 72 h. With both techniques, cell adhesion, growth and migration can be controlled dynamically after the cells started to grow on the substrate. This opens new possibilities: we believe the key to control the development of topologically controlled neuron networks or more complex co-cultures is the combination of passive surface modifications and active control over the surface properties at any time of the experiment.

1. Introduction

Experimental techniques in biomedical engineering often involve the use of patterned substrates to study fundamental cellular processes. The patterned surfaces are engineered to specifically influence cell adhesion, outgrowth, migration, organization and tissue development by providing alternating surface properties in the micrometer range. In principle the cells are seeded on prefabricated substrates patterned either with different materials e.g. metals, metal oxides or organic molecules which preferably do not change their properties during the experiment. Such passive substrates are patterned by normal photolithography techniques for spatial arrangements of different solid materials [1], protein-aqueous materials [1–5], polyelectrolytes [6,7] or self-assembled monolayers (SAMs) [8,9]. Photolithography in combination with laser ablation was also reported [10]. Another method, "μ-contact printing" developed by Whitesides et al., uses a microstructured stamp made of poly-(dimethylsiloxane) (PDMS) [11]. This printing technique is mostly used to transfer patterns of poly(L-lysine) (PLL) [12–17] or extracellular matrix proteins such as laminin, fibronectin to a flat substrate. Instead of proteins, synthetic molecules consisting of substrate binding sites and specific cell adhesive peptide sequences (e.g. RGD, IKVAV, etc.) can be printed onto solid materials [18–20]. Another approach for patterning cells is to provide a 3D structure on a surface were cells can attach and grow inside or along the structure. Merz and Fromherz used lithographically patterned SU8 polyester structures to guide individual snail neurons [21,22]. Topographical effects of smaller structures such as silicon pillars and micron-sized holes on neuron growth were also investigated [23,24]. There were also attempts to culture neurons in microfluidic platforms allowing directed growth of neurites from their cell bodies [25] or pattern them at low densities for further differentiation [26]. It has also been tried to trap the neurons mechanically in parylene cages positioned on electrode sites of a multielectrode array (MEA) chip. The cell body is hold in place by the cage, while the neurites are free to grow into the surrounding area [27].

All these methods provide a prefabricated passive patterned surface for cell cultures. Nowadays, the challenge shifts towards more complex structures built of different cell types or to

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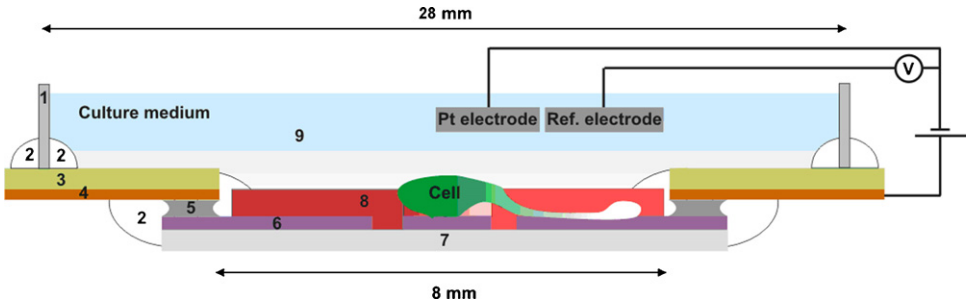

Fig. 1. Schematic drawing of the assembled neurochip unit. PMMA ring (1) is glued with PDMS (2) to the printed circuit board (3) to form a liquid chamber for the cell culturing. PCB copper paths (4) are electrically connected with silver epoxy resin (5) to the ITO structure (6) on the SiO_2 substrate (7). PDMS (2) seals the remaining gaps between epoxy silver glue (5) and SU8 structure (8). Platinum and chlorinated silver wires immersed in the culture media serve as counter and reference electrodes, respectively.

build topographically controlled neuron networks towards further understanding how the brain works or to study the effects of neuron active drugs in vitro [28]. It is therefore crucial to develop more versatile patterned surfaces with active control over the surface properties at any point in time of the experiment. These so called stimuli responsive surfaces [29] switch their surface properties triggered by photons, enzymes, temperature or electric potentials. Sarig-Nadir et al. used a laser to change the local properties of a PEGylated fibrinogen hydrogel to allow the outgrowth of a neuron in the desired direction [30]. Another way to realize a dynamic control over the surface during cell culturing was introduced by Yeo and Mrksich. They used alkane-thiol SAMs on a gold surface exposing cell adhesive RGD ligands attached to an electroactive group [31]. Upon applying an electric potential the electroactive group with the ligand is released resulting in the detachment of adherent fibroblasts from the surface. Kaji et al. described a method using a conductive probe to generate an HBrO (oxidizing agent) from the Br^- containing solution in the vicinity of an albumin coated surface [32,33]. Locally, the albumin was turned cell adhesive and permitted the cells to grow along the trace of the conductive probe. Zhao et al. showed that protein and cell repulsive OEG-terminated SAMs become cell adhesive upon exposure to electro-generated Br_2 [34]. Fan et al. used pluronic polymer coated electrodes and was able to control the protein adsorption by tuning the voltage bias on the microelectrodes [35]. Li et al. described a method to pattern cells by prior electrochemical desorption of SAMs in localized areas defined by a microfluidic system [36]. Cells could also be patterned by simple electrochemical desorption of PEG silane SAMs from gold [37] or ITO electrodes [38].

In this paper we present a combined approach by providing a 3D structure and electrical control over the neuron adhesion sites. We were able to control the neurite outgrowth by desorption of the protein resistant coating electrically [39]. To our knowledge it is the only attempt so far to electrically control the outgrowth of single neurons. Furthermore, we show a new technique using only small pulsed currents to actively inhibit the migration of myoblasts onto ITO electrodes. We believe the combination of the two presented patterning techniques described in this paper is an attractive possibility to engineer topographically controlled neuron networks or to control the built-up of more complex tissues consisting of multiple cell types.

2. Materials and methods

2.1. Neurochip microfabrication

2.1.1. ITO electrodes

Microscopy cover slides (24 mm × 24 mm × 0.17 mm) were sputter coated with 50 nm indium-tin-oxide (ITO) layer at the Institute of Microtechnology, University of Neuchâtel, Switzerland. A wet etching mask was produced by common photolithography with Shipley S1805 photoresist (Rohm & Haas, Germany) on the ITO cover slide before etching in 3 M HCl. After etching, the photoresist was removed by immersion in pure acetone for 5 min, followed by rinsing in isopropanol, ethanol and H_2O.

2.1.2. SU8 Microstructure

The cover slide with the etched ITO electrodes was put on a heating plate at 150 °C for 20 min to remove adsorbed water and improve the SU8 adhesion. Then, an SU8 layer (GM1040, Gersteltec, Switzerland) was spin coated in two steps onto the substrate to achieve a more homogeneous thickness. The first layer was 4 μm thick (1000 rpm, 40 s) and the second layer 10 μm thick (400 rpm, 40 s), whereby each layer was consequently soft baked (10 min, 65 °C; ramped to 95 °C (ΔC = 2 °C/min); 30 min, 95 °C). The combined SU8 layers were exposed for 32 s through a chromium mask (Delta Mask, Netherlands) in a Karl Süss X380 mask aligner and post-baked with the same temperature profile as used for the softbaking. The SU8 structure was developed in 2-methoxy-1-methylethyl acetate (PGMA) for 2 min, rinsed with isopropanol and dried at ambient air, before hard baking the SU8 at 135 °C for 2 h on a hot plate (Fig. 2A).

2.1.3. Neurochip assembly

Different parts were assembled to form a whole unit providing a liquid chamber for cell culturing and macroscopic electrical contacts (Fig. 1). The ITO electrodes were therefore connected with silver epoxy resin to corresponding copper paths on a printed circuit board (PCB). To form the liquid chamber, a poly(methyl methacrylate) (PMMA) ring was glued with PDMS to the PCB top side. After curing the silver epoxy and PDMS at 90 °C for 3 h, remaining gaps between neurochip and PCB were sealed with PDMS and cured again. A platinum wire was immersed into the buffer solution or culture media as counter electrode and a chlorinated silver wire as reference electrode. The +1.9 V used to remove the PLL-*g*-PEG was always applied versus the reference electrode.

2.1.4. Cell culture, staining, imaging

NG108 neuroblastoma cells (LGC, France) were cultured in a growth medium (DMEM without pyruvate, 10% fetal bovine serum, HAT supplement) at normal cell growth conditions (37 °C, 5% CO_2). Once the neurons adhered to the neurochip the culture medium was exchanged to serum free differentiation media (DMEM without pyruvate, HAT supplement, 500 μM dibutyryl cAMP). In all cell culture medium 1% penicillin–streptomycin was used to avoid bacterial growth. All cell culture medium and supplements were bought from Invitrogen, Switzerland.

At the desired time point of an experiment the cells were fixed with 4% formaldehyde in PBS for 30 min and permeated in PBS (1% BSA, 0.1% Triton X-100) for 30 min. The cells were then