TRI-COMPARTMENT CHIP WITH MICROELECTRODE ARRAY AND DR1-GLASS GROOVES FOR NEURONAL CELL ALIGNMENT

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ABSTRACT

We report a modular cell-culture chip that combines three modules, which we have studied separately earlier: custom-designed microelectrode array (MEA) technology, a microfluidic polydimethylsiloxane (PDMS) module with three cell culture compartments separated by microtunnels, and nano-scale grooves made of light-responsive Disperse Red 1 molecular glass (DR1g) to guide neuronal cell growth across the middle compartment over the embedded electrodes. The functionality of the chip was verified by culturing human-induced pluripotent stem cell (hiPSC) -derived cortical neurons in the chip for 33 days. The DR1g grooves were found to align the axonal growth and we were able to record neuronal activity in all three compartments. The chip can be utilized in a large variety of axon biology-related studies, as it offers axonal guidance, possibility to produce controlled axonal damage and to measure axonal activity.

KEYWORDS

Axons, Azobenzene, Cell Alignment, Microelectrode Array, Neuronal Cells, Polydimethylsiloxane, Surface Relief Grating

INTRODUCTION

Many of the central nervous system (CNS) related diseases are characterized by axonal damage and dysfunction. Axonal degradation is a complex process, and its full mechanisms are yet to be uncovered, therefore making axonal degradation research highly important in disease studies. Some of the neurodegenerative diseases affected by axonal degradation are for example Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD). [1, 2] The challenge in studying axons by using microelectrode arrays (MEAs) and advanced organ-on-chips is that the axons tend to grow to random directions. Thus, means to guide the axonal growth to wanted direction, e.g., to grow over the electrodes such that the electrical activity of the axons could be measured, are needed.

In our earlier studies we have shown that nano-grooves made of light-responsive azobenzene-based Disperse Red 1 molecular glass (DR1g) can be used for guiding, e.g., human neuronal axons to follow the topography at the bottom of a cell culture well [3]. The obvious benefit of implementing microstructured light-responsive materials compared with other cell-guiding methods such as cell-favoring coatings or grooves etched to the MEA substrate or to the passivation layer is that they allow not only creating but also erasing the grooves using light in non-harmful manner [4]. Both can be done when the cells are already added onto the substrate, which is not possible, e.g., if the grooving is done by etching or using photolithography.

We have also introduced a microfluidic polydimethylsiloxane (PDMS) module with three cell compartments separated by microtunnels (Fig. 1) and have successfully demonstrated its usage in experiments performed for neuron-oligodendrocyte [5] and neuron-cardiomyocyte [6] co-cultures.

To enable electrophysiological recording or stimulation in addition to microscopic characterization, we have earlier proposed a concept [7] where a custom-designed MEA is integrated on each microtunnel-separated cell culture compartment including DR1g grooves in the middle compartment. The DR1g grooves were used for guiding the axons to grow across the middle compartment (the "axon" compartment) and over the electrodes. Now we have implemented the proposed concept (Fig. 2 and 3) and here we verify its functionality with human-induced pluripotent stem cell (hiPSC) -derived cortical neurons.

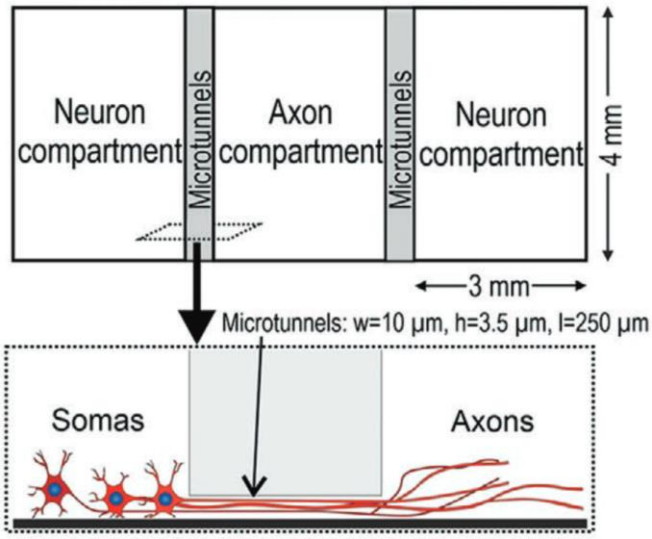

Figure 1: Schematics of the microfluidic PDMS module of the chip and its use in neuronal cell studies [3].

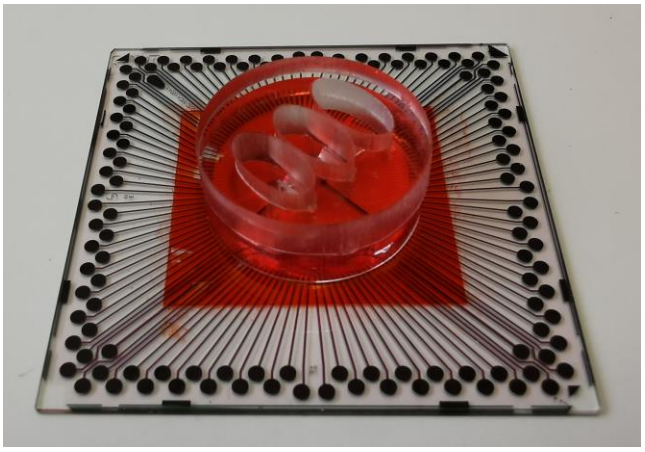

Figure 2: Photograph of the tri-compartment chip including a 120-electrode MEA, a microfluidic PDMS module containing three cell culture compartments separated by microtunnels and grooves for guiding axonal growth in a DR1-glass layer (red).

In the implementation phase, the major challenges to solve were how to remove the DR1g from the top of the electrodes without damaging the electrodes and how to secure leak-free adhesion of the PDMS module on the underlaying DR1g-coated MEA. Solving the latter issue with a thin additional PDMS layer between the DR1g

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layer and the PDMS module meant that the cells are actually cultured on PDMS surface instead of the earlier-proven DR1g surface or typical silicon nitride (SiN) surface of the MEA. So, in this study, we had to pay attention also to protein coating to secure best the adhesion of cortical neurons.

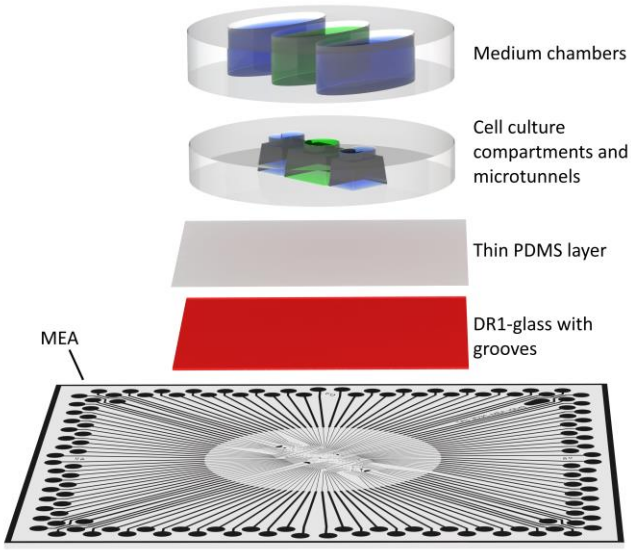

Figure 3: Explosion image of the modular tri-compartment chip.

MATERIALS AND METHODS

MEA fabrication and characterization

A custom-designed MEA acts as the base for the chip. The MEAs are compatible with the 120 electrode MEA format of Multi Channel Systems. The chip contains three compartments: two end compartments ("neuron compartments") and an axon compartment between them. In the neuron compartments, there are 26 electrodes (Ø = 30 µm) in 6-8 × 4 format and 400 µm pitch. Under the micro-tunnel sections, there are 4 mm long, 30 µm wide line electrodes that go below all the tunnels. In the axon compartment, there are 57 electrodes (Ø = 30 µm) in 5-8 × 9 format with a pitch varying from 400 µm to 650 µm. In addition, in all three compartments there is a large half-circle-shaped grounding electrode and three other larger electrodes intended for potential stimulation or insulator layer degradation evaluation. The MEAs were fabricated in-house by the process described in [8]. Briefly, 49 mm × 49 mm × 0.7 mm borosilicate glass slides (g-materials) were used as a substrate. The conducting layer was e-beam evaporated titanium, thickness 200 nm. PECVD deposited (@350°C) 500 nm SiN acted as the passivation layer and the electrodes and the contact pads were coated with a 300 nm layer of ion-beam assisted e-beam deposited titanium nitride (TiN) to decrease the impedance and the noise level of the electrodes and to improve the mechanical properties of the contact pads against sharp contact pins.

Impedances of the electrodes at 1000 Hz were measured using MEA-IT120 impedance measurement device (Multi Channel Systems). Dulbecco's Phosphate Buffered Saline (DPBS) w/o Magnesium, w/o Calcium (Biowest) was used as the electrolyte.

DR1g grooves and opening the electrodes

To create the grooves, the central surface area of the MEA delimited by the contact pads was spin-coated with 480±20 nm DR1g (Solaris Chem, 5% w/v in chloroform, 1500 rpm, 30 s), and 60 nm PDMS (Sylgard184 Dow, 10:1 prepolymer silicone elastomer base:curing agent diluted in n-hexane to get a 1 wt% solution) layers. The PDMS layer was spinned at 6000 rpm for 150 s and cured at 55 °C for 1.5 h. The PDMS layer was found necessary to secure leak-free adhesion of the microfluidic PDMS module with microtunnels and compartments on the MEA, because the adhesion of the PDMS module directly to DR1g was not found sufficient to prevent leakages in initial tests.

Removal of DR1g and the thin PDMS layer on top of the electrodes was done by laser ablation using an in-house-built multipurpose laser system. Several laser power levels and number of pulses were tested (not discussed in more detail in this paper) until one 8.86 µJ pulse per 30 µm electrode using 515 nm femtosecond laser (Pharos Ligh Conversion 20 W) was found sufficient. For opening long tunnel electrodes and grounding electrodes, a 342 nm laser wavelength and a scanning mode was used. The impedances of electrodes were re-measured after the ablation the same way as before the ablation to confirm that the electrodes did not get damaged by the removal process and that the removal was complete.

After that, a two-beam interference setup implementing a 488 nm continuous-wave laser (Coherent Genesis CX488-2000) with circular polarization and intensity of 300 $mWcm^{-2}$ was used for creating sinusoidal grooves with 1 µm period onto the DR1g layer in the axon compartment.

Microfluidic PDMS module

The microfluidic modules were fabricated from PDMS using hybrid moulds that were produced using SU-8 lithography and stereolithography based 3D printing [5, 6]. 3D printing was used to increase the height of the cell compartments in the moulds larger than what is possible with traditional photolithography. The neuron compartments are 4 mm wide, 3 mm long and 2.2 mm high, and the axon compartment is 4 mm wide, 5 mm long and 2.2 mm high. The compartments are spaced 250 µm apart and are connected to each other by 40 microtunnels being 3.5 µm in height and 10 µm in width. The compartments are made open with punching, and the medium chambers placed on top of the cell culture compartments are fabricated with laser cutting from 4 mm thick PDMS [6]. The PDMS module was permanently bonded onto the PDMS layer of the MEA using O_2-plasma to complete the chip. Explosion image of the chip is presented in Fig. 3.

Cell experiments

After the assembly of the chip, cell compartments were coated with human-recombinant laminin-521 (Biolamina) because it supports cell adhesion onto the PDMS surface on the MEA. HiPSC-derived cortical neurons [9] were plated to the neuron compartments (40 000 cells per compartment). Cell culture medium (BrainPhys maintenance media, that is BrainPhys base media supplemented with 20 ng/ml BDNF, 10 ng/ml GDNF, 500 µM cAMP and 200 µM AA) was changed three times a week. Once a week 100µg/ml LN521 was added to the medium in the ratio of 1:1000 to improve the cell adhesion. Finally, the cells were fixed after 33 days in culture.

RESULTS AND DISCUSSION

As the functionality of the three modules have been separately verified earlier, the main challenges in their integration were i) to secure the cell adhesion on the thin PDMS layer, ii) to provide leak-free adhesion of the PDMS module to the MEA, and iii) to ensure the removal of DR1g and PDMS from the top of the electrodes without damaging the electrodes. Thanks to the laminin coating, neurons attached and grew well on the chip and in the axon compartment the axonal growth was more unidirectional in the grooved chips than in the non-grooved chips (Fig. 4).

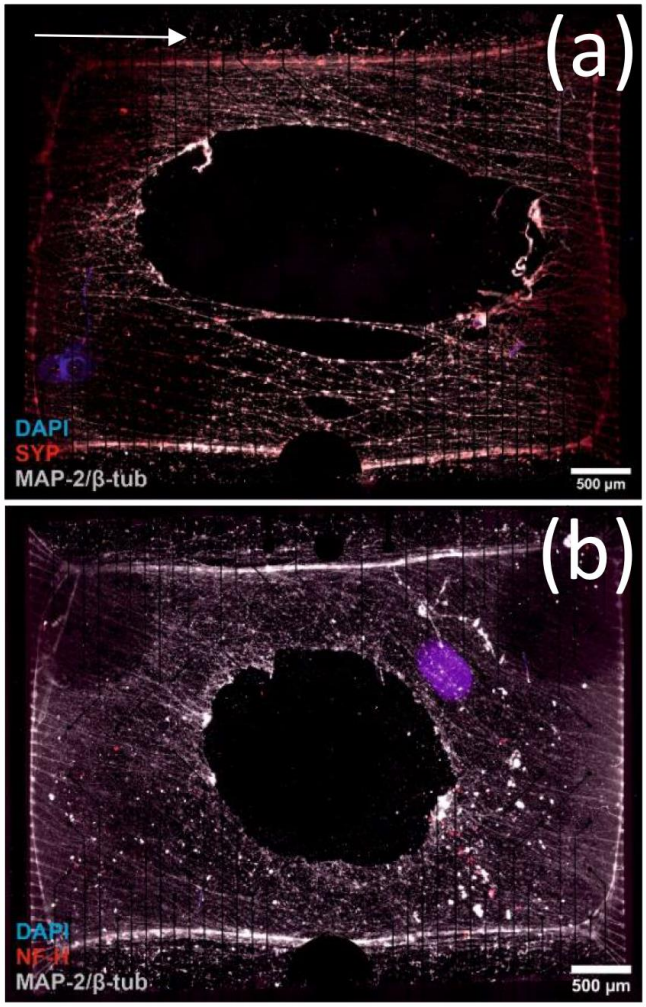

Figure 4: ICC image of the axon compartment of (a) grooved and (b) non-grooved chip. Axons in the middle of the compartment degraded and got removed during fixing after four weeks of culture. Direction of the grooves is indicated with white arrow.

The cells started to degrade only after 33 days. Because the degradation occurred in all samples, the reason was concluded not to be caused by the DR1g but either due to the wear of the laminin or because the axons had no target organ to reach out to. We were able to record neuronal activity with the MEA especially from the neuron compartments where the neuronal somas located (Fig. 5). A lower cell activity in the axon compartment is explained by the fact that there were mainly axons in that compartment. Successful cell activity recordings prove that the laser removal of DR1g and PDMS did not harm the electrodes. This observation was supported by impedance measurements made for the electrodes while optimizing the laser parameters. Histograms of the impedances of 30 µm electrodes of 13 MEAs show that in the majority of the electrodes the impedances after laser ablation were between 100 kΩ and 200 kΩ as before DR1g and PDMS coating. In some cases, the impedance has even lowered which indicates that the laser may have removed also some oxidation from the electrodes. On the other hand, roughly 25 % of the electrodes have an increased impedance, which we assume to indicate a partial removal of DR1g and PDMS. We assume this to be caused by not fully optimizing the laser power, and by varying accuracy of focus and alignment of the laser beam in the system used. However, for the aims of this study, we estimated that the reached results were enough to confirm the viability of the ablation process and therefore decided to leave the further optimization to future studies. We are confident that by doing more iteration rounds and/or using a commercial, more stable laser system the ablation success rate can be easily increased even further. In the future, the DR1g technology makes it possible to modify cell substrate topology by e.g. erasing the grooves and re-creating them at a desired time instant [4], which makes such experiments as studying sudden axonal damage possible.

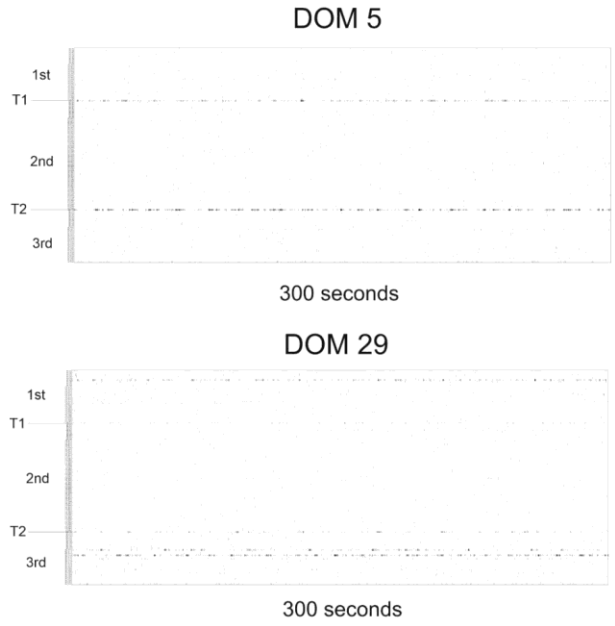

Figure 5: Raster images showing the activity on days on MEA (DOM) 5 and DOM 29."1st", "2nd", and "3rd" are the three compartments with 26, 57 and 26 electrodes in each and "T1" and "T2" are long line-shaped electrodes located in the tunnels between compartments.

CONCLUSION

Grooved DR1g on MEA oriented axonal growth and neuronal cells showed electrical activity, proving the functionality of the chip. The presented chip solves the challenge of guiding axons to grow to a desired direction after passing the tunnels from another compartment. With integrated electrodes, the grooved chip enables several neuronal applications such as axonal dysfunction in Parkinson's disease and axonal damage in stroke.

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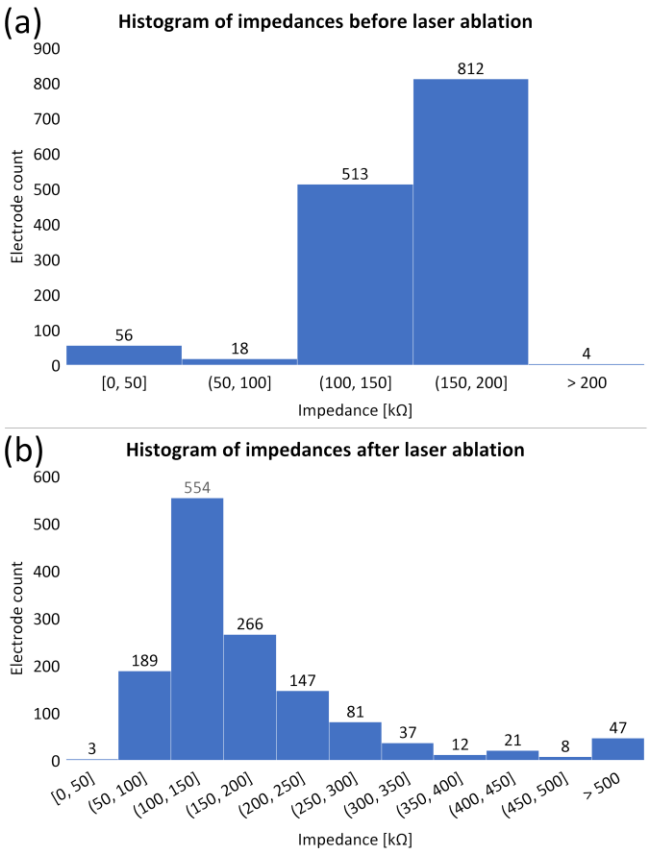

Figure 6: Histograms of impedances of 30 µm electrodes of 13 MEAs (a) before DR1g and PDMS coating and (b) after laser-ablation.

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