Design and fabrication of microchannel and cell culture scaffolds for neural guidance and enhanced optical accessibility of neural networks *in vitro*

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Abstract

Physical confinements and chemical cues are commonly applied to substrate surfaces to control neuronal attachment and growth. Temporal physical confinements can be produced with transparent polydimethylsiloxane (PDMS) microchannel tiles which stick reversibly to flat surfaces. In the present study, PDMS microstructures were overlaid on poly-D-lysine (PDL) and laminin-coated microelectrode arrays (MEAs) to let axons and dendrites grow through electrode-crossing microchannels. Three days after cultivation, neurites stated to grow into the channels to result in dense networks of axons and dendrites at 14 days *in vitro* (DIV), from which signals could be recorded at 17 DIV. The results showed that PDMS microchannels successfully force neurites to grow along defined pathways. The reversible bond between PDMS structures and the MEA surface makes such tiles a compatible tool for optical manipulation or staining studies.

1 Introduction

Low density culture and substrate patterning strategies are the methods of choice for controlling and tracing network connectivity on MEAs [1]. Several methods have been reported in the literature for patterning surfaces to control neuronal attachment and growth including chemical and topographical cues [2]. However, mechanical confinements usually require the substrates to undergo various pretreatments. And in most cases, the resulting structures are irreversibly fixed to the substrate. Finally, it is still difficult to combine such pre-patterned substrates with chemical patterning approaches [3]. We are therefore interested in exploring alternative concepts for the combined, but independent chemical and topographic patterning of networks.

1.1 Combination of chemical printing and physical confinement

Cell culture substrates such as Petri dishes or MEAs can be pre-patterned chemically by *e.g.*, polylysine (PL) through microcontact printing (µCP). Given that PDMS sticks to almost all flat surfaces without any glue, microchannel sheets with through-holes can be reversibly placed on these pretreated substrates. By design, the microchannels will thus be connecting individual through-holes laterally. In such setup, neurons can be seeded from above into these freely accessible through-holes, which simultaneously serve as cell culture microwells. Neural processes then have no other choice but to grow in these wells and interconnect through the microchannels. Depending on

the placing of the microchannel sheets with respect to the electrode matrix, different cell compartments may become accessible to MEA electrophysiology and/or optical manipulation in neural regeneration studies.

2 Methods

2.1 PDMS µ-channel molding

PDMS µ-channel tiles with overall heights of 200 µm were fabricated by replica-molding from bi-level SU-8 µ-structures on a 4" silicon wafer (*Fig. 1*). These structures featured four 200 µm-high oval-shaped reservoirs and 30 µm diameter columns for through-hole generation. Reservoirs were connected by an 8 × 8 matrix of 40 µm wide and < 70 µm high channels. Columns were located at the channel crossings. PDMS (Dow Corning, Sylgard 184) pre-polymer and curing agent was mixed (10:1), poured on an epoxy copy of the SU-8 master and cured at room temperature for 2 days applying slight pressure with help of a clamp. The resulting PDMS µ-channel tile was perforated by oval shape reservoirs with interconnecting channels.

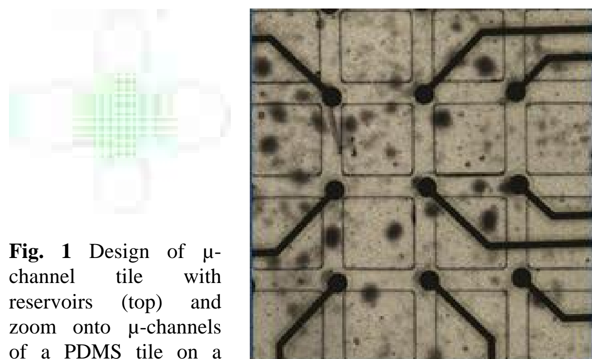

Fig. 1 Design of µ-channel tile with reservoirs (top) and zoom onto µ-channels of a PDMS tile on a 30/200 MEA (right).

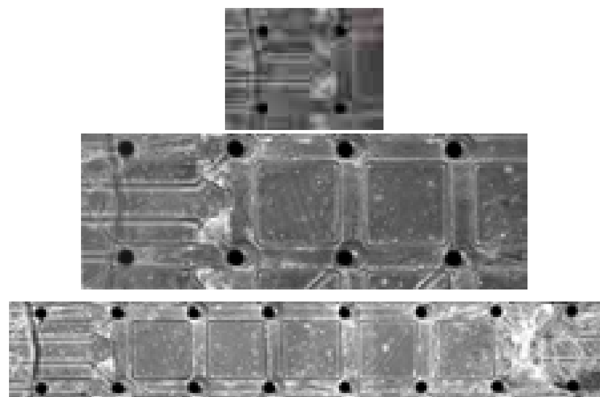

Fig. 2. Neurites are growing through 40 µm wide PDMS microchannels (5 DIV, 9 DIV and 14 DIV).

2.2 Cell culture

MEAs where hydrophilized by oxygen plasma (2 min, 50 W, 0.3 mbar O_2) and their surface coated with 0.1 mg/ml PDL and 0.1 mg/ml laminin (20 µl), which was allowed to dry. MEAs were washed twice with sterile ultrapure water and dried. To align the PDMS tiles with the MEA electrodes, a droplet of sterile water was used to temporarily decrease the physical adherence of PDMS to the MEA substrate (*Fig. 1*). After complete water evaporation, the reservoirs were filled with cell suspension (20 µl; 6000 cortical cell/µl, rat E18). Cells were allowed to settle on the PDL/laminin coat for 40 min in the incubator before 1 ml of serum-free medium was added (NBM, B27, Ala-Glu, Pen/Strep). Cultures were stored in a standard incubator (5% CO_2, 37 °C, 95% RH).

3 Results

Microscopic pictures were taken daily from 3 DIV onward to monitor neurite growth through the µ-channels (*Fig. 2*). At 3 DIV and 5 DIV, some neurites had reached the first channel junctions. The number of neurites in each channel and the number of channels with neurites increased with age (6 DIV - 14 DIV). After 14 DIV, axons from neurons in one reservoir had extended to the other reservoir (≤ 1.5 mm). The picture series furthermore revealed that neurites tended to change their positions within channels (*Fig. 2*). At 17 DIV, first signals could be recorded (not shown). This was late compared to unconfined control networks from which activity was recorded at 8 DIV. Because the overall channel cross section was large, some cells migrated into and within the channels and produced very low-density networks with growing axons or dendrites.

The shortest distance between two reservoirs was 1400 µm which showed that some axons can grow in PDMS channels over long distances. This will allow

4 Conclusions

PDMS µ-channel tiles were successful in guiding axons and dendrites along specific paths over MEA electrodes. Because the number of neurites per channel was rather low, their growth could be monitored easily by time-lapse photography. Some axons had crossed the shortest distance of 1400 µm between two reservoirs, which showed that even *in vitro* axons can grow over long distances. The presented PDMS µ-channel tiles will allow users to manipulate growing neurites by optical tools and evaluate their growth in detail. The tiles can be removed non-destructively after neurite ingrowth to perform any kind of classical optical manipulation or staining study. Current work focuses on the effect of different channel cross sections on growth rate and signal shape.

Acknowledgement

IIT intramural funding of this research line is greatly appreciated.

References

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