Polymer Chip for AmperometricDetection of Neurotransmitter Release from Single Cells

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Polymer Chip for Amperometric Detection of Neurotransmitter Release from Single Cells

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A polymer microchip for recording of neurotransmitter release from living neuroactive PC 12 cells was developed. Electrochemical studies show that exocytotic dopamine signals can be recorded using conducting polymer poly(3,4-ethylenedioxythiopene) (PEDOT) microelectrodes. Microchips were made by combining two injection molded TOPAS parts. Nickel mold inserts for the injection molding process were electroformed from Si master templates containing the microfluidic channel structures.

Objective

Secretion of signaling molecules by cellular exocytosis is a key mechanism in many communication processes between living cells. Single exocytotic events can be studied by electrochemical sensing techniques using microelectrodes. The goal of this project is to develop a polymer microchip where neuroactive cells can be easily trapped near microelectrodes and to investigate the potential of the conductive polymer poly(3,4-ethylenedioxythiophene) (PEDOT) to work as microelectrode material for electrochemical detection of the neurotransmitter dopamine.

Chip design and cell clamping

Microchips were made by combining two injection molded TOPAS parts. The upper part contained microfluidic channels and openings while the lower part was covered with PEDOT microelectrodes. Living neuroactive PC 12 cells were successfully trapped at a specified site inside a chip using a so-called mouse hole configuration.

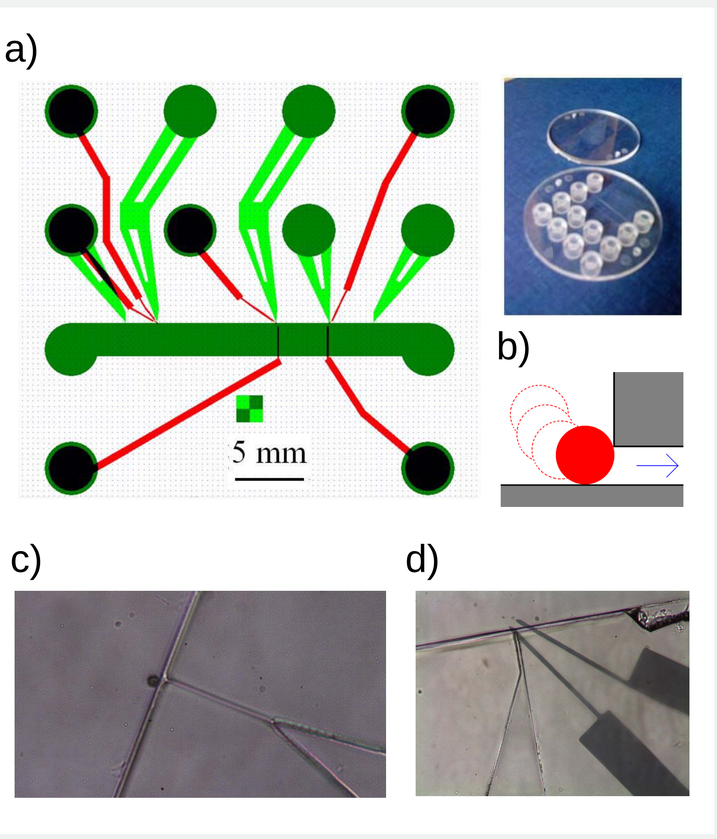

Figure 1: a) Photomask designs for microchip fabrication. Microfluidic channels are shown in green (dark green: 50 µm deep channels, light green: 5 µm deep channels). Electrodes are shown in red. The large circles are chip openings. b) Illustration of cell clamping at a “mouse hole”. The blue arrow indicates suction. c) Living PC12 cells can be trapped at the channel junctions. This microscope image shows a trapped polystyrene microbead of approximately same size as PC12 cells (r = 7,5 µm). d) Microscope image of two PEDOT electrodes aligned with a channel junction.

Pedot microelectrodes

The conductive polymer PEDOT was used as electrode material. Cyclic voltammetry experiments showed that the neurotransmitter dopamine can be electrochemically detected on PEDOT electrodes (fig 2). By making the electrode surface sufficiently small noise levels below 2 pA can be reached. This is an acceptable noise value for measuring current peaks from single exocytotic events.

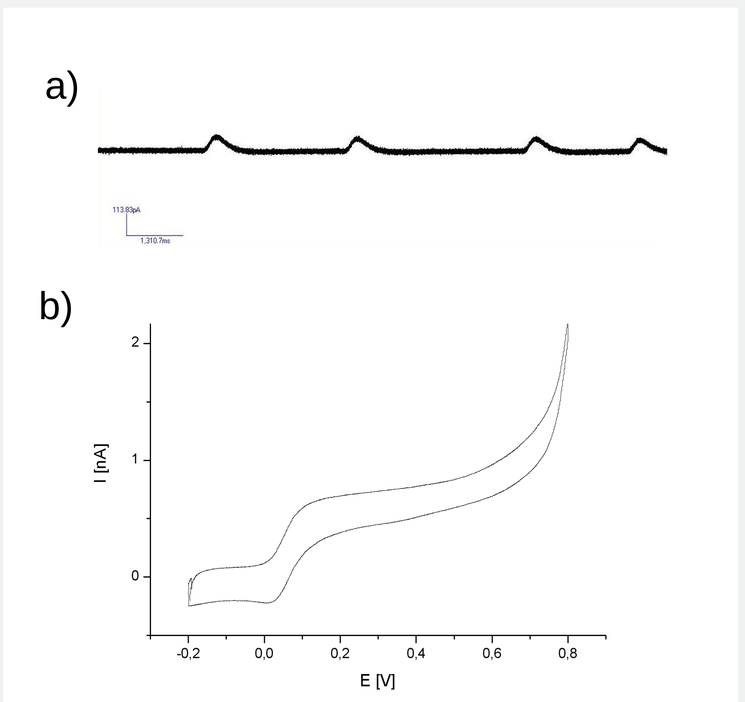

Figure 2: a) Current trace recorded at a PEDOT microelectrode. Spikes result from oxidation of dopamine being ejected into the phosphate buffer through a micropipette (d=2 microns) situated close to the electrode. Cyclic voltammogram showing the oxidation and reduction of dopamine on a PEDOT electrode. Dopamine was diluted in PBS buffer. Concentration: 1 mM, scan rate: 20 mV/s, electrode dimensions: 4 µm X 700 µm.

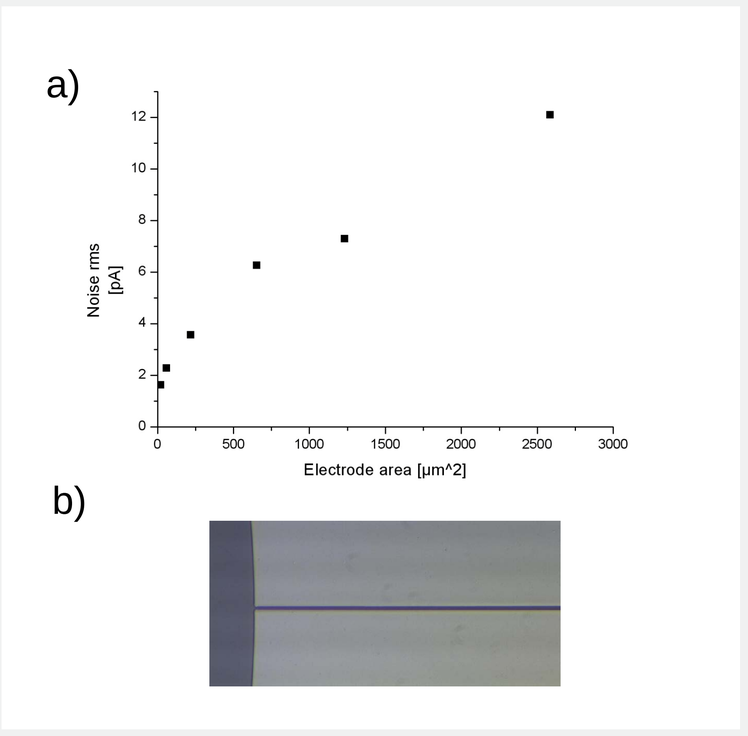

Figure 3: a) Noise versus electrode area. The noise was calculated as the root mean square noise of a current trace with the electrode being exposed to phosphate buffer. b) Microscope image of a 2 micron wide PEDOT electrode.

Perspectives

One of the limitations of current technology used for measuring exocytotic signals electrochemically is the low through-put. We hope with this polymer chip platform to develop a device that is both cheap and capable of automatically measuring exocytotic activity of several cells in one sample.

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