Conductive Polymer Microelectrodes for on-chip measurement of transmitter release from living cells

S. T. Larsen*, M. Matteucci* and R. Taboryski

*Department of Micro- and Nanotechnology,
Technical University of Denmark, DTU Nanotech, Building 345E,
DK-2800 Kongens Lyngby, Denmark

ABSTRACT

In this paper, we present techniques to trap a group of neuronal cells (PC 12) close to band microelectrodes and quantitatively measure cellular transmitter release. Different trapping approaches were investigated including coating of electrodes by layers enhancing cell attachment and by pressure driven cell trapping inside closed chip devices. Conductive polymer microelectrodes were used to measure transmitter release using electrochemical methods such as cyclic voltammetry and constant potential amperometry. By measuring the oxidation current at a cyclic voltammogram, the concentration of released transmitter molecules could be estimated.

Keywords: pedot, conductive polymer, exocytosis, neurotransmitter, amperometry

1 INTRODUCTION

Exocytosis is the principal means of communication between neuronal cells and connected to several central nervous system disorders. In this process, neurotransmitters stored in intracellular vesicles are released to the surroundings by fusion of the vesicle membrane with the cell membrane. Exocytosis can be evoked in cultured neuronal model cell lines (we use PC 12) by exposing the cells to a high concentration of potassium. A range of neurotransmitters oxidize at a certain potential and can be detected as a current signal, by holding an electrode at a constant potential higher than the oxidation potential.

The idea of using conductive polymer electrodes for electrochemical measurement of transmitters is new [1] and is here utilized by using photolithographically patterned Poly(3,4-ethylenedioxythiophene):tosylate (Pedot:tosylate) microelectrodes. A wide range of transmitters were shown to oxidize readily on these electrodes in a recent study by the authors of this work [2].

Much research has been focused on measuring single exocytotic events on carbon fiber microelectrodes or thin film metallic microelectrodes [3], [4]. On the other hand, little work has been done on measuring transmitter release from large cell groups, although this method is experimentally easier and has a high potential in drug screening applications [5]

In this paper, we investigate some novel approaches of trapping a population of cells close to a microelectrode and keeping them fixed during buffer exchange. This is done in all-polymer chip devices. Further, we make use of conductive polymer electrodes with excellent electrochemical properties. We demonstrate how cellular release of transmitters can be measured using constant potential amperometry at these electrodes.

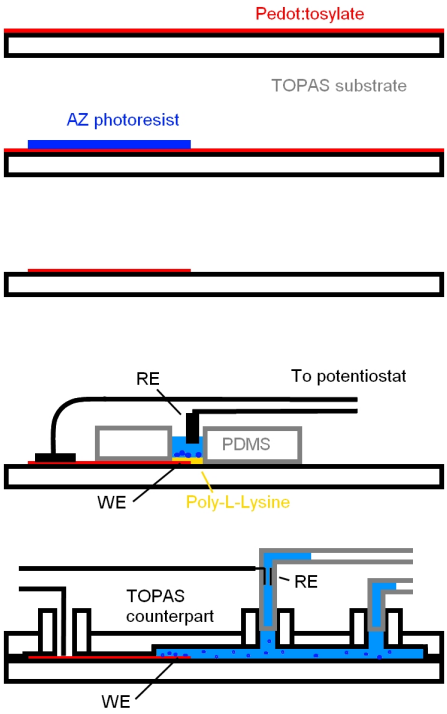

Figure 1: Fabrication of Pedot:tosylate microelectrodes and chip integration. After application of the Pedot:tosylate, patterning is done with UV lithography using AZ resists and reactive ion etching. Two different chip designs have been used in this work, by bonding to either a well structure in PDMS or an injection molded TOPAS counterpart with microchannels. Abbreviations: WE – Working Electrode, RE – Reference Electrode.

2 FABRICATION

Micropatterned conductive polymer electrodes were fabricated on flat injection molded TOPAS 5013 (TOPAS Advanced Polymers GmbH) substrates as described in detail earlier [2] and shown in figure 1. Two different methods of integration of electrodes into chip devices have been used in this work.

In the first approach, the electrode substrate was bonded to a well shaped PDMS part. The electroactive area of the electrode was defined by the area protruding into the well. Attachment of cells to the electrode could be achieved by incubating the electrodes with a 0,01 mg/ml Poly-L-Lysine or Collagen solution for 1 hour prior to cell experiments. A 2-electrode configuration was used with a Ag/AgCl reference electrode placed in the cell buffer.

The second approach which is shown in the bottom drawing in figure 1, involved thermal bonding of the TOPAS substrate to an injection molded TOPAS counterpart. In order to fabricate samples by means of injection molding, Nickel shims with channel design are fabricated with standard UV-LIGA techniques. Parts are then injection molded from TOPAS using an Engel Victory 80/45 Tech hydraulic injection molding machine. Before bonding, the two TOPAS parts need to be aligned by eye with an optical microscope. Thermal bonding is done at 120° C and 10 kN for 5 minutes. Microchannels can be accessed through luer holes in the injection molded top part. A VEMA 8 channel pressure regulator (Festo) was used to control and move fluids inside the chip. A Ag/AgCl electrode was constructed to fit inside a luer connector and was used as reference electrode.

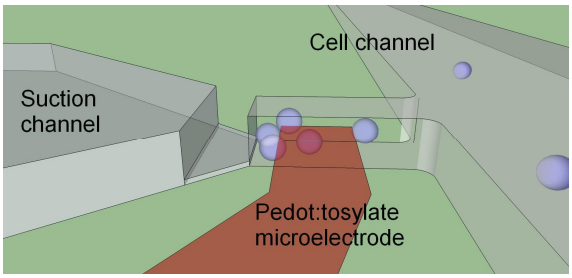

Figure 2: Illustration of cell trapping approach where cells are trapped inside a 20 µm x 30 µm x 100 µm chamber due to suction from a separate suction channel. A 2 µm high slit section ensures cells being left in the chamber.

3 CELL TRAPPING

3.1 Cell chamber approach

One of the approaches used in this study to trap cells is shown in figure 2. By applying suction through a 3 µm high slit, cells are pulled into a 30 µm x 20 µm x 100 µm box from the adjacent cell channel. A Pedot:tosylate microelectrode can be fabricated and aligned to protrude into the cell chamber from one side. A microscope image of a final chip is shown in figure 3 (right). The electrode shown here is 50 µm wide which makes the electroactive area 50 µm x 30 µm.

Passage 12 rat pheochromocytoma (PC 12) cells were used to test the cell trapping technique. After applying a small vacuum pressure (around 50 mbar), cells started to flow in and fill the chamber. After filling approximately ¼ of the chamber the pressure had to be increased to attract more cells. A final pressure of 500 mbar was used to fill up the chamber as seen in figure 4.

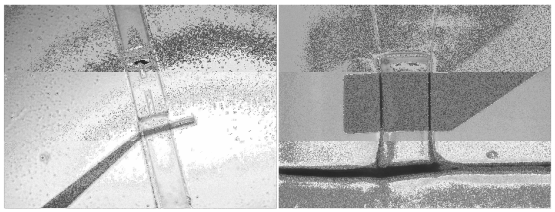

Figure 3: Microscope images of injection molded and thermally bonded polymer chip devices. Left: 1-dimensional trapping technique with Pedot:tosylate electrode placed right before shallow channel region. Right: Cell chamber trapping technique as shown in figure 2.

3.2 One-dimensional channel with slit

Another cell trapping approach consisted of only one large cell channel with a 100 µm slit region approximately half way between the inlet and outlet openings. A Pedot:tosylate electrode was fabricated to cross the large channel on the inlet side of the slit (fig 3, left side). Although this approach seems to be simpler, it did not perform as well as the cell chamber approach in terms of channel filling and cell capture.

3.3 Cell trapping by "sticking" surfaces

The method of trapping cells at an electrode by coating the electrode with a "cellophilic" biomolecule was successfully applied by Liu et al [6] and used to measure transmitter release from single cells at a DLC:N/ITO electrode. In this work, we coated Pedot:tosylate electrodes with Poly-L-Lysine and Collagen (both acquired at Sigma Aldrich). These compounds are frequently used to coat cell culture substrates. Both surfaces exhibited superior cell attachment compared to bare polymer substrates even though the time allowed for attachment is limited to less than 20 minutes due to cell viability. Poly-L-Lysine seemed to be slightly better than Collagen. Further, the additional layer did not compromise the good electrochemical

characteristics of Pedot:tosylate electrodes. Once cells are trapped on and around a band electrode, transmitter release can be triggered simply by exchanging the buffer solution with a buffer containing a high potassium concentration. Figure 5 shows a microscope image of a large group of PC 12 cells sedimented on a Pedot:tosylate band microelectrode.

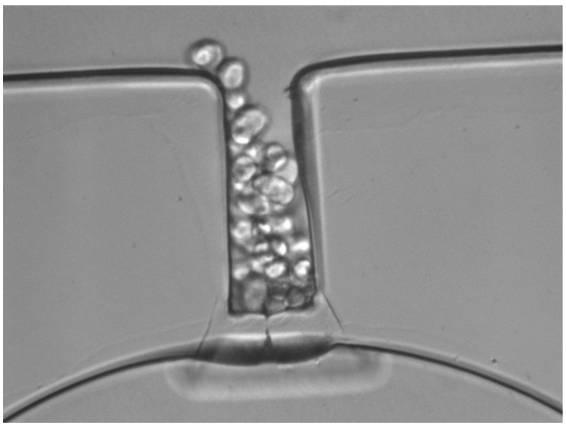

Figure 4: Microscope image showing the filling of a micro-chamber by PC 12 cells

.

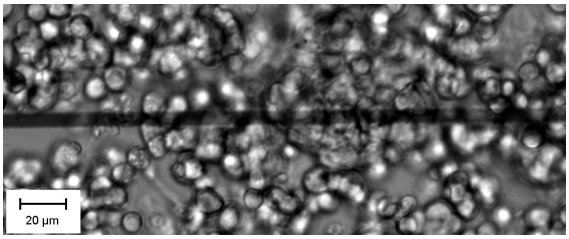

Figure 5: Sedimentation of PC 12 cells on a 7 µm wide Poly-L-Lysine coated Pedot:tosylate electrode.

4 ELECTROCHEMICAL MEASUREMENTS

Before cell experiments, we tested the Pedot:tosylate electrodes by performing slow scan cyclic voltammograms with electrodes immersed in varying dilutions of dopamine in PBS buffer. The result is shown in figure 6. As expected, cyclic voltammogram step height is proportional to dopamine concentration. For small concentrations of dopamine a voltage independent oxidation current is reached.

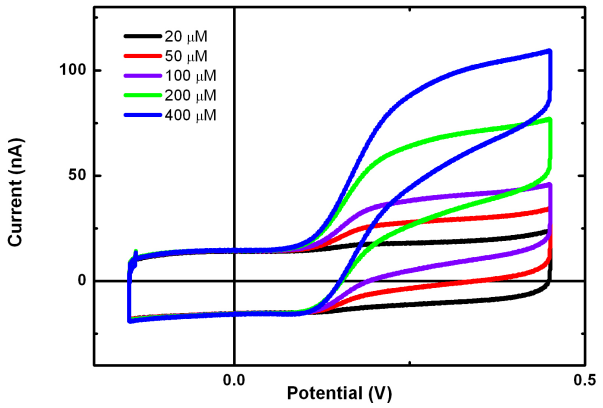

Figure 6: Cyclic voltammograms showing the oxidation of Dopamine on a Pedot:tosylate microelectrode. Dopamine was diluted at different concentrations in PBS. Scan rate: 10 mV/s, Potential referenced to Ag/AgCl

Next, we coated a Pedot:tosylate band electrode with Poly-L-Lysine and let a population of PC 12 cells sediment on the surface. After sedimentation, the buffer used to transport the cells could be exchanged with fresh physiological buffer without moving the attached cells. A cyclic voltammogram performed with the cell-covered electrode immersed in fresh PBS showed no sign of oxidizing compounds (black line, figure 7). By exchanging the buffer with a similar buffer containing higher potassium concentration (105 mM), transmitter release from the PC 12 cells could be detected as a step on the cyclic voltammogram (blue line, figure 7). The step occurs at the same potential (~150 mV) as seen for dopamine dilutions (fig 6), which strongly indicates that dopamine or other catecholamine release is observed.

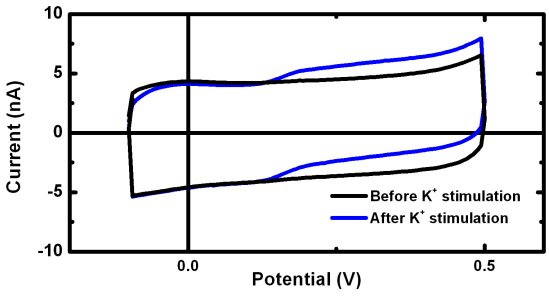

Figure 7: Cyclic voltammograms performed at a Pedot:tosylate electrode covered with Poly-L-Lysine and PC 12 cells. The black voltammogram was measured before K^+ stimulation and the blue voltammogram right after K^+ stimulation.

Since the oxidation current, measured as the step height in figure 7, is directly proportional to the concentration of oxidizing molecules, we can calculate the concentration of

released transmitter molecules. The quasi-steady state oxidation current at a band electrode is given by the following equation:

$$i_{qss} = \frac{2\pi nFlDC}{\ln(64Dt / w^2)} \quad (1)$$

where C is the bulk concentration, n the number of electrons in the reaction, F Faraday's constant, t the time after oxidation onset, D the diffusion coefficient, and the dimensions of the band electrode are given by the width w and length l. In figure 7, the measured oxidation currents of the dopamine dilution voltammograms from figure 6 are plotted together with values calculated using eq. 1. This result shows, that eq. 1 is valid for oxidation of dopamine at Pedot:tosylate and we can now use it to estimate the concentration of transmitter molecules released from PC 12 cells and electrochemically detected in figure 7. Using the electrode dimensions 12 µm x 1560 µm, time 15 s and the measured oxidation current 1,2 nA, we obtain a calculated transmitter concentration of 10 µM around the electrode.

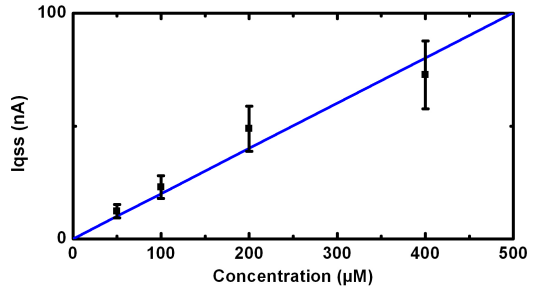

Figure 7: Measured quasi-steady state oxidation currents corresponding to voltammograms in figure 6, and theoretical values (eq. 1, blue line)

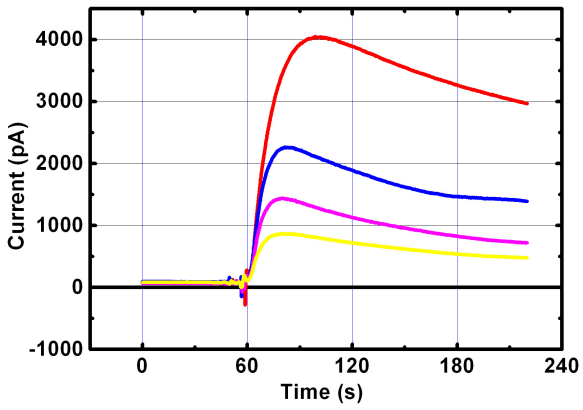

Figure 8: Amperometric current traces at a Pedot:tosylate electrode covered with Poly-L-Lysine and PC 12 cells. The cells were alternately exposed to a potassium rich and low potassium buffer. The largest response corresponds to the first K^+ stimulation, while subsequent responses decrease.

Finally, we measure the increase of released transmitter molecules from a group of PC 12 cells, by applying a constant potential to the Pedot:tosylate microelectrodes and measuring the current before and after potassium stimulation. In figure 8, the current traces corresponding to 4 subsequent potassium stimulations at the same group of PC 12 cells are shown. Between stimulations, the cells are given rest periods in low potassium buffer for 4 minutes. The decreasing current responses could be interpreted as the cells depleting their transmitter reservoirs due to heavy release.

5 CONCLUSION

In this paper, we measured transmitter release from large groups of neuronal cells by using electrochemical detection at Pedot:tosylate conductive polymer microelectrodes. We investigated three different cell trapping approaches. Trapping cells inside a cell chamber or on top of a sticking coating layer gave good results. Finally, we measured transmitter release from PC 12 cells at Pedot:tosylate electrodes using cyclic voltammetry and constant potential amperometry. Oxidation currents could be related to dopamine concentrations by a simple formula, and concentration of released transmitter could be estimated.

6 ACKNOWLEDGMENTS

This work is supported by the Danish Council for Strategic Research through the Strategic Research Center PolyNano (grant no. 10-092322/DSF)

REFERENCES

[1] S. Y. Yang, B. N. Kim, A. A. Zhakidov, P. G. Taylor, J.-K. Lee, C. K. Ober, M. Lindau and G. G. Malliaras, Adv. Mater., 23, H184-H188, 2011

[2] S. T. Larsen, R. F. Vreeland, M. L. Heien, and R. Taboryski, Analyst, 137 (8), 1831 - 1836, 2012

[3] R. M. Wightman, J. A. Jankowski, R. T. Kennedy, K. T. Kawagoe, T. J. Schroeder, D. J. Leszczyszyn, J. A. Near, E. J. Diliberto and O. H. Viveros, Proc. Natl. Acad. Sci., 88, 10754-10758, 1991

[4] C. Spegel, A. Heiskanen, L. H. D. Skjolding, J. Emneus, Electroanalysis, 20, 680-702, 2008

[5] H.-F. Cui, J.-S. Ye, Y. Chen, S.-C. Chong, and F.-S. Sheu. Anal. Chem, 78, 6347-6355. 2006

[6] X. Liu, S. Barizuddin, W. Shin, C. J. Mathai, S. Gangopadhyay, and K. D. Gillis. Anal. Chem., 83, 2445–2451, 2011

[7] A. J. Bard and L. R. Faulkner, John Wiley & Sons Inc, 2001