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Versatile electrochemical sensor for cell culturing

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Abstract Culturing of organotypic brain tissues is a routine procedure in neural research. The visual inspection of the medium is the only way of determining the state of the tissue. At the end of culturing, post-processing techniques such as HPLC can be used to measure the concentration of the secreted metabolites in the waste products. Continuous measurements would enable improved monitoring as compared to the end-point assay. Here, we developed a sensor system capable of real time measurements of the analytes directly secreted from the tissue. The presented system can be readily integrated in the standard procedures allowing for better assessment of the progress of the culturing.

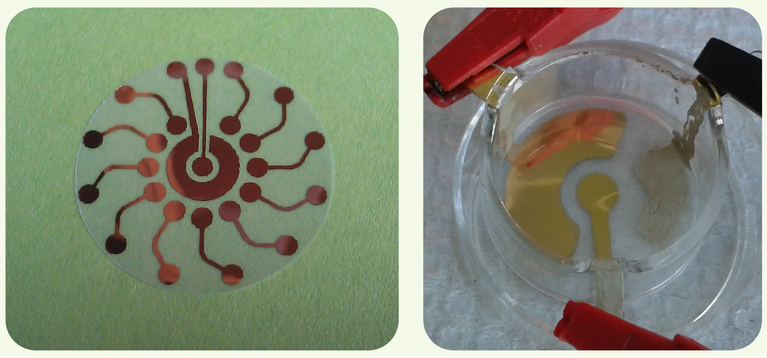

Figure 1 Membrane electrodes fabricated on two different types of membranes. Left: Gold electrodes on a flat polycarbonate membrane using E-beam evaporation. Right: Gold and silver electrodes fabricated on a cell culture insert using sputtering.

The sensor system was initially developed for monitoring of cells and tissue cultures but has lately been considered for, and tested in, a wide range of applications. Some of these include pathogen detection and integration in microfluidic devices for sample preparation.

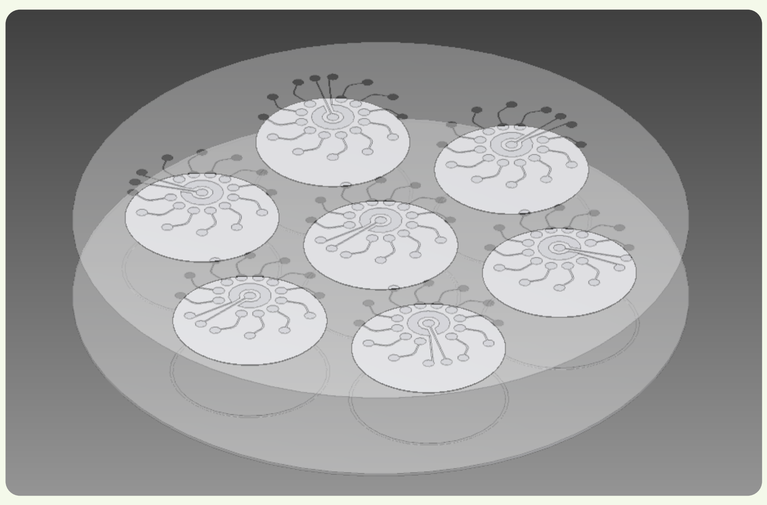

Figure 2 Illustration of setup for electrode fabrication on flat membranes using E-beam evaporation. The bottom layer is a thin piece of PMMA with alignment marks for the membranes. In the middle are the membranes and on top is a thin PMMA shadow mask with the electrode pattern.

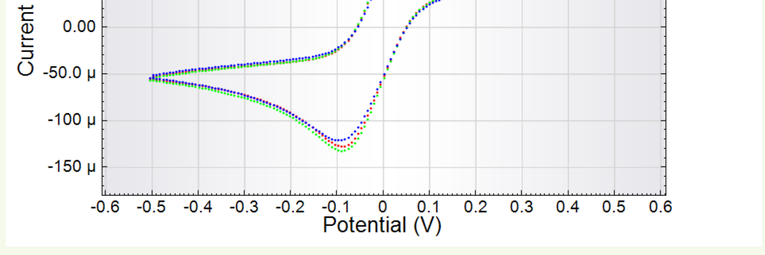

Figure 4 Representative CV performed in 10 mM ferri ferrocyanide in PBS. The CVs were performed at scan rates of 500 mV/s, 400 mV/s, 300 mV/s, 200 mV/s, 100 mV/s and 50 mV/s in the listed order. Identical measurements have been performed on numerous electrodes on a number of different membranes with similar results. A cleaning procedure was tested but was seen to have no positive effect.

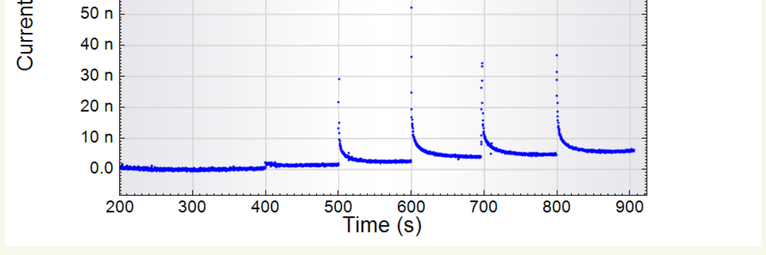

Figure 6 To test the stability of the membrane electrodes during longer measurements CVs with 100 consecutive cycles were performed in 10 mM ferri ferrocyanide at 6 different scan rates: 500 mV/s (red) , 400 mV/s (green), 300 mV/s (blue), 200 mV/s (black), 100 mV/s (magenta) and 50 mV/s (forest green). The electrodes appear to be stable during long term measurements, especially for the lower scan rates.

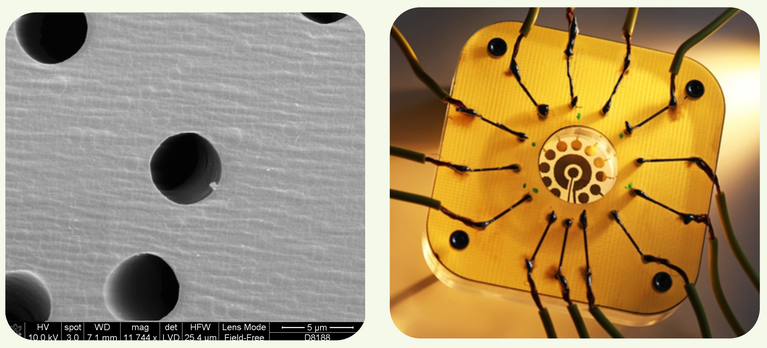

Figure 3 Left: SEM picture of polycarbonate membrane with 200 nm gold deposited by E-beam evaporation. The pore size is unchanged after gold deposition. Right: Device for electrochemical characterization of membrane electrodes. The membrane holder was designed in two versions in order to test the potential for integrating the membrane electrodes in fluidic devices. In the first version the membrane was bonding in the device and in the second version the membrane was clamped in the device using screws and an O-ring. Both were fitted with a costume PCB board for connection to a potentiostat.

Figure 5 To test the long term stability of the membrane electrodes during storage CVs were performed in 10 mM ferre ferrocyanide in PBS on a single electrode over a longer period of time. The graph shows three CVs that were performed on the same electrodes 2 weeks apart. The red curve is the first measurement and the green and blue is the second and third respectively. As can be seen the signal does not change noticeably over time.

Figure 9 Amperometric measurement of addition of dopamine to a nitrogen purged PBS solution. Dopamine was added every 100 s from 300 s. The concentration was as follows 0 M, 100 pM, 1 nM, 10 nM, 100 nM, 200 nM, 500 nM. Det measurement was made as a proof of concept and has not been optimized in any way. However a baseline correction was performed in Nova 1.10.

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