A cell-on-paper system for the study of secretion

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ABSTRACT

A simple and generally applicable system was designed to detect and quantify electroactive molecules released from cells grown on paper.

KEYWORDS: electrochemical methods, cells-on-paper, exocytosis

INTRODUCTION

Detecting and quantifying secreted molecules is a critical requirement for reliably analyzing physiological phenomena in cells. Neurotransmission is here of specific interest because of the biological and medical significance of this phenomena, as well as the electroactive nature of many neurotransmitters. If the molecule of interest can be oxidized or reduced, electrochemistry becomes a powerful technique, requiring only affordable and simple instrumentation. A simple and versatile electrochemical setup, allowing for rapid, reliable and quantitative measurements from a wide range of cell samples, would therefore be beneficial.

EXPERIMENTAL

Here, a hybrid microfluidic/ electrochemical system is described for the detection of dopamine (DA) from a population of 5 x 10^5 PC12 cells cultured on a patch filter paper [1]. The cells were grown onto the surface of the paper patch using an extracellular matrix gel. The system itself consists in two parts (Figure 1A): first the sample chamber made of two similar parts that are clamped together to maintain the sample in the flow of a microfluidic system (Figure 1B), and second the detection chamber (Figure 1C), which is to contain a three-electrode electrochemical setup, with an Ag|AgCl reference electrode (RE), a Pt working electrode (WE) and a counter (CE) electrode formed by a syringe needle. These electrodes were encased in a syringe needle to facilitate the handling, as shown in Figure 2.

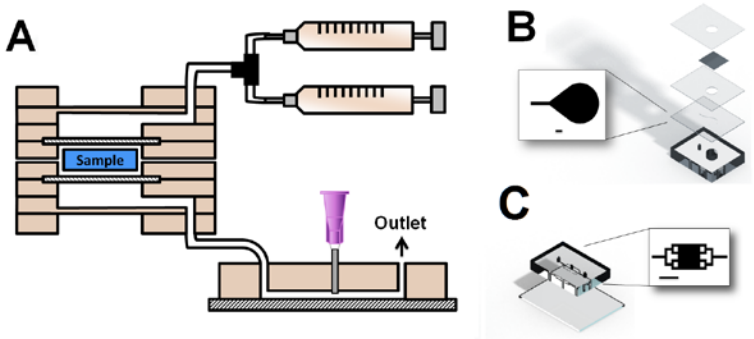

Figure 1. Description of the device. A- Scheme of the assembled cell-on-paper system, showing the input syringes actuated by a syringe pump and the sample and detection chambers. B- Schematic of one of the two identical parts that together compose the sample chamber. C- Schematic of the detection chamber

RESULTS AND DISCUSSION

The system was fully characterized electrochemically and calibrated. It is also shown that the molecule used for stimulating the cells, acetylcholine (ACh), does not itself elicit any electrochemical response within the error of the measurement.

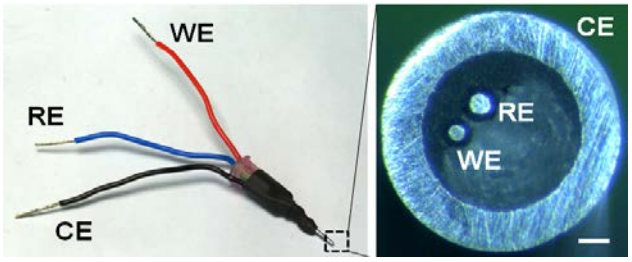

Figure 2. Picture of the electrochemical electrodes encased into a syringe needle with, on the right, a micrograph of the polished tip of the needle, showing the three electrodes (scale bar is 100 μm).

Furthermore, using fluorescence microscopy, it is demonstrated that the cells adhere to the surface of the paper and are partially integrated into the bulk of the material, and are not flushed away by the flow of buffer in the sample chamber (Figure 3).

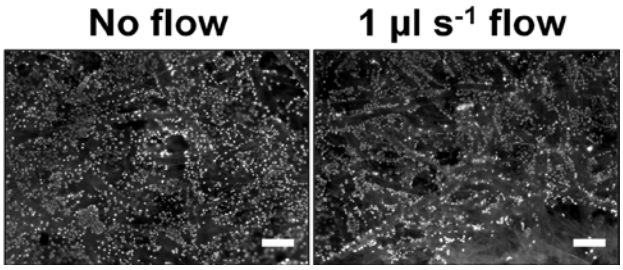

Figure 3. Fluorescence imaging (DAPI) of PC12 cells deposited in the paper before (left) and after applying a flow of 1 μl s-1 for 10 minutes, showing good immobilization of the cells in the paper. The scale bar is 100 μm.

Once the system is characterized and calibrated, the cell-seeded patch of paper is placed in the system, and DA release is triggered by injecting ACh. An increase in current, indicative of DA release, was observed for the cell-seeded patches (Figure 4), but not for the control case, where no cell is present in the chamber. Furthermore, a ~30 s delay before the onset of DA release was measured, in agreement with the slow response of PC12 to ACh stimulation [2].

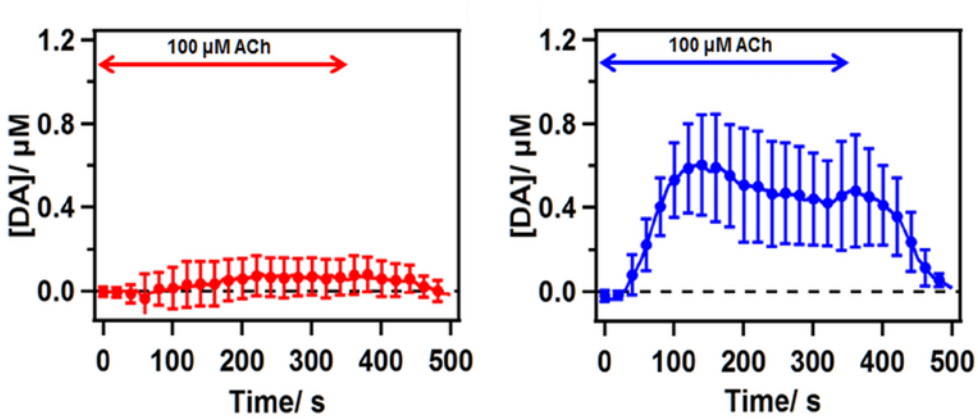

Figure 4. Dopamine concentration profiles obtained from the system for controls where no sample is placed in the system (left, n= 9) and when a paper patch seeded with half a million PC12 cells is inserted in the chamber (right, n=7). The data is average ± SD.

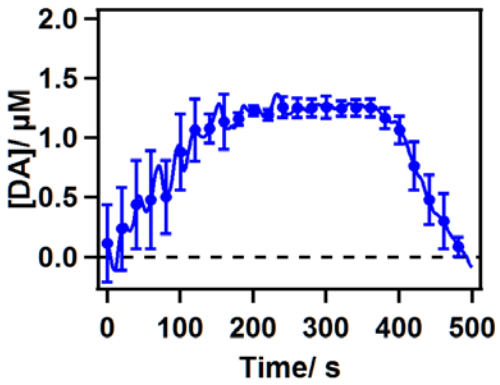

Figure 5. Dopamine concentration profile obtained from paper patches seeded with half a million PC12 and pre-incubated with 100 µM of the drug L-DOPA (n=3). The data is average ± SD.

Finally, the effects of L-3,4-dihydroxyphenylalanine (L-DOPA) were also studied. This drug is commonly used in the treatment of Parkinson's disease and increases the amount of DA contained in the cells [3]. After pre-treatment with L-DOPA (Figure 5), the cells were found to release higher levels of DA (~ +150 %).

CONCLUSION

Overall, the results obtained with this system were in good agreement with data previously published at the single cell level, thus demonstrating the validity of our approach for quantitative chemical analyses at cells, tissue or artificial cell constructs, such as organs-on-a-chip. It also further strengthens the applicability of electrochemistry for specific projects were continuous detection is critical.

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