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Amperometric fluidic microchip array sensing device for nitric oxide determination in solution

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Abstract

In this paper, we report on the first use of an amperometric fluidic microchip array for the examination of nitric oxide in solution. The array chip is composed of 36 working platinum electrodes on a glass substrate. The electrodes have a diameter of 50 μm and are separated by 500 μm. The array chip is integrated within a flowing cell to obtain a fluidic-type sensing device. Two preliminary tests were performed. The first one consisted in assessing the fluidic set-up by using potassium ferrocyanide as test analyte. The second test was aimed at achieving the modification of the surface of the working electrodes by electrodepositing nickel tetrasulfonated phthalocyanine and Nafion® layers to show that the fluidic sensing device can be adapted to the analysis of nitric oxide in solution.

Keywords: Sensor; Nitric oxide; Compact device; Electrode array; Fluidic system

1. Introduction

The measurement of nitric oxide (NO) is important for direct examination of its regulatory roles in biological systems since it participates in the control of major cell functions [1–3]. In view of the complexity of the functions and interactions of NO, a proper investigation of its production and kinetics, in response to cell modulation by chemicals of biomedical relevance, necessitates the online monitoring of its generation and extracellular spatial distribution. Extracellular measurement of NO efflux is now well documented and, so far, the electrochemical methods are recognized as the only analytical way to do so [4]. Indeed, electrodeposited nickel porphyrin or phthalocyanine films on carbon and graphite microfibers brought a substantial contribution to the development of efficient electrochemical NO-microsensors [4]. Surprisingly, most of the electrochemical microsensors reported in the literature during the last decade are made using carbon or graphite fibers as electrode material, despite the heterogeneity of the various sources of used materials and the difficulty in reproducing and renewing their surfaces, while platinum and gold, which offer the apparent facility of having easily reproducible materials with various shapes and geometries of electrodes, have been used only rarely. Recently, we and others have successfully reported on the use of platinum and/or platinized needle type and disk arrays electrodes for the sensitive and selective determination of NO production from cultured cells [4–8].

Nowadays, the major important criteria in the making of an NO electrochemical sensor are the design and fabrication of electrode arrays having various sizes and arrangement. The still increasing sophistication involved in this type of activities allows having diverse approaches, especially when using platinum. Our interest in designing electrochemical sensing array-based devices for biological NO production from cultured cell layers encourages us to explore the possibility of developing a new advance integrating microelectrode arrays within a simple flow-through device. Such a flow-through approach could help in bringing close information on the extracellular diffusion and dynamics of NO as soon as it is formed from one particular part of the cell culture. Also, one may expect collecting information on NO formation and dynamics flowing local drug delivery on a restricted part of the cultured cells. Thus, this study reports on preliminary results obtained with an array of multiple working Pt microelectrodes on glass substrate inserted within a flowing

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platform. The tests were undertaken to show the feasibility of this set-up concept for the determination of two simple electroactive analytes, $Fe(CN)_6K_4$ and NO in solution.

2. Experimental

Planar thin-film Pt microdisk array consisting of 36 microelectrode arrays was fabricated on a glass substrate using standard thin-film technology, as previously reported [9]. The microelectrodes have a diameter of 50 μm each and are separated by 500 μm (Fig. 1a and b). The passivation layer consists of a 400-nm-thick PECVD Si_3N_4. The electrode array is integrated within a flowing platform manufactured by Central Research Laboratories Limited (Heathrow, UK). Fig. 1c shows the scheme of the flow-through device. In brief, each microelectrode can be addressed individually via the purpose-built jig, which provides contact pins for each electrode contact pad, and an acrylic cell. The cell is clamped onto the glass chip, using a silicone gasket to form a liquid seal, so-called chamber.

A peristaltic IP-ISMATE pump was used for fluidic tests, with silicone tubes allowing having a flow rate of 18 mL/h.

Electrochemical experiments were carried out at room temperature, in aerobic conditions, using PC-controlled potentiostats (VA-10 npi model (Germany) and EGG 263 A and EGG VersaStat II models from Princeton Applied Research (USA)), with data acquisition and analysis software. External Pt counter and (pseudo-)reference electrodes (either Ag/AgCl or Pt) were used in all measurements.

All solutions were prepared using ultra-pure Milli-Q water (Millipore© UP system). NO introduction into the electrochemical cell was done by adding aliquots of a NO-saturated phosphate buffer solution (PBS) to the deaerated electrolyte.

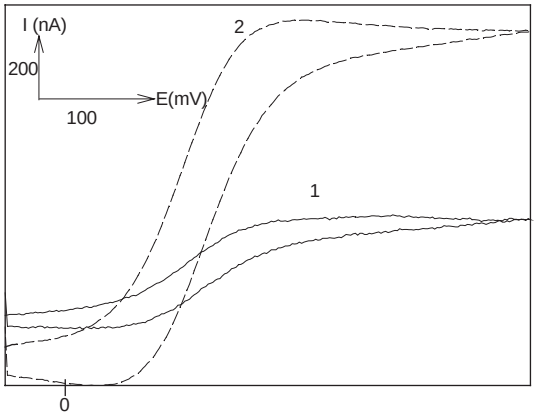

Fig. 2. Cyclic voltammograms of 0.05 M $Fe(CN)_6$ phosphate buffer solution (pH=7.4) at one connected microelectrode at the entrance side (1) and three connected microelectrodes at the exit (2) (scan rate=100 mV/s).

To produce the saturated NO solution (NO ≈ 2 mmol/L at 20 °C [10]), an oxygen-free buffer solution was purged with pure NO gas (Aldrich) for 20 min and kept under NO [11]. NO was always passed through a 0.1 M aqueous KOH solution to remove impurities. Standards were freshly prepared before each experiment and kept in a glass flask with a rubber septum at 0 °C.

All chemicals were reagent grade and used as-received.

3. Results and discussion

3.1. Test of the flow-through device

In a first approach, we have tested the possibility of assessing the flowing detection through the whole device by using $Fe(CN)_6K_4$ as simple electroactive analyte. Two groups

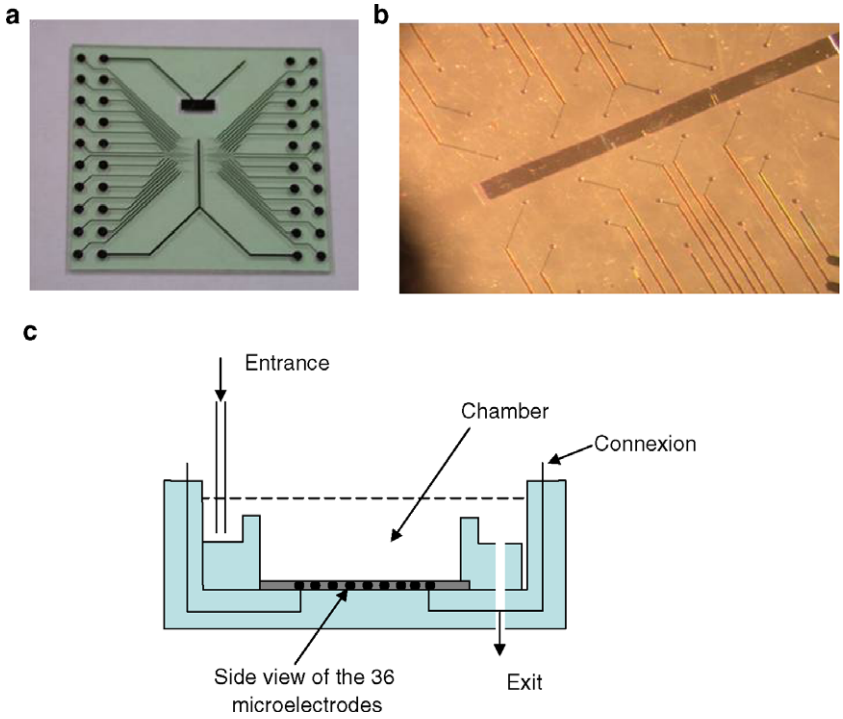

Fig. 1. Photos of the cell-chip (a), zoom on ultramicroelectrodes (b), and transverse cut scheme of the fluidic device (c).