INTEGRATED MULTI-ELECTRODE FLUIDIC NITRIC-OXIDE SENSOR AND VLSI POTENTIOSTAT ARRAY

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ABSTRACT

Nitric Oxide (NO) serves as an important intercellular messenger in the human body, and is mechanistically involved in vascular disease, stroke, chronic hear failure, and epilepsy. We present a multi-electrode NO measurement sensor with integrated microfluidics. An array of carbon-based electrodes detect the spatial and temporal diffusion profile of NO in a PDMS laminar fluidic channel. The array interfaces to a multichannel potentiostatic VLSI system recording the amperometric output in real time, with five orders of magnitude in dynamic range over four scales down to hundreds of picoamperes. The integrated sensor-fluidic-VLSI system is expected to serve as a powerful tool to study the diffusion mechanism of NO under different fluid flow paradigms.

1. INTRODUCTION

Nitric Oxide (NO) has been linked intrinsically to the mechanisms of a number of debilitating disease states like stroke, ischemic injury and heart attack [1]. A number of these phenomena are related to alterations in the flow of blood to the affected areas of the body. NO is the most potent vasodilator known, due to its action in the endothelial cells of arterial vessels [2]. Understanding its mechanism of action through its release, diffusion and uptake in the endothelial layer of blood vessels could provide invaluable insights into the disease states themselves.

The measurement of biological NO signals poses multiple challenges. Primarily, *in vivo*, NO has a fleeting lifetime ($t_{1/2}$ <250 ms), and therefore any sensor technology must be capable of capturing this brief signal. The concentrations of NO present *in vivo* are also small, typically less than 1 μM, except under extraordinary conditions. These demands call for a technology capable of sensing small concentrations of NO rapidly, and from distributed locations simultaneously. While optical techniques and indirect chemical detection are popular, electrochemical oxidation at an electrode surface could provide the best profile of features for such a detection system.

A number of NO sensors based on different forms of carbon have been reported, and have been used successfully *in vivo*. George *et al.* [3] reported the design and fabrication of an NO sensor array using carbon ink screen printing. Sensors based on electrochemical oxidation-reduction (redox) reactions respond to a broader class of neurochemicals. These include dopamine, an important neurotransmitter that plays an important role in disorders such as Parkinson's disease. The carbon sensor array was used for monitoring the concentration of electroactive neurotransmitters from distributed locations in the brain.

This work was supported by NIMH R21-MH-062444 and R21-MH-065296, NSERC 261606-0, ONR N00014-99-1-0612, and the Whitaker Foundation. Chips were fabricated through the MOSIS foundry service.

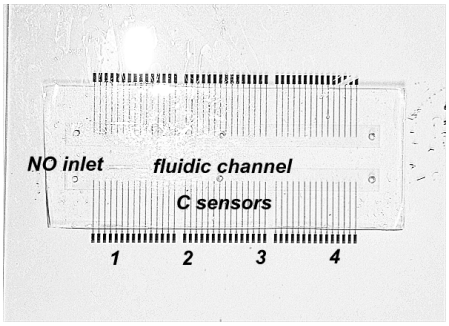

Fig. 1. *Microfabricated multi-electrode NO sensor array with integrated microfluidic channels.*

Here we present a microfabricated sensor array with integrated fluidics, and interface this device with a multi-channel VLSI potentiostatic data acquisition system [4] for the simultaneous detection of NO, dopamine and other neurochemicals in multiple locations in a fluid flow channel.

2. MULTI-ELECTRODE MICROFLUIDIC NO SENSOR

Microfabrication techniques have been employed to construct arrays of microelectrodes on silicon substrates [5, 6], or on glass culture plates [7]. Sophisticated semiconductor fabrication techniques have been employed to devise arrays of microsensors [8], and, in some cases, analog interface circuitry [9].

These electrode arrays measure electrical neural activity. Continuous measurement of the activity of biochemical signaling molecules is now possible using electrochemical sensor technology. The integrated microsensor arrays presented in [3] are capable of mapping the spatial and temporal distribution of neuronal messengers or neurotransmitters such as NO and dopamine.

In the present work, distributed biochemical signal acquisition is combined with microfluidics to study NO transport phenomena in a simulated vascular flow environment. The integrated fluidic multi-electrode NO sensor is shown in Figure 1. Two microfluidic channels are shown, each interfacing with 45 carbon-based

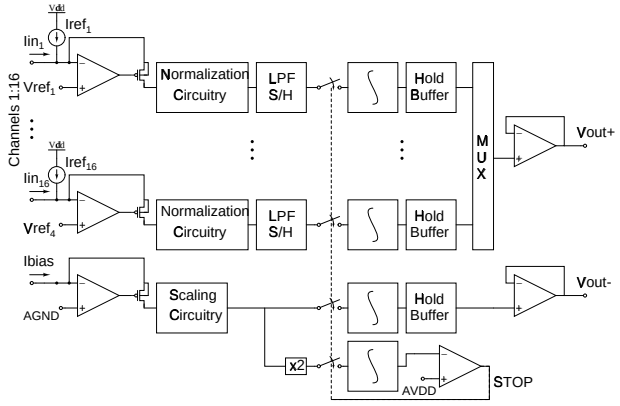

Fig. 2. *Block diagram of the integrated track-and-hold potentiostat.*

NO sensors. Electrodes are 100 μm wide and consist of a base layer of Ti (50nm), Au (250nm) and C (250nm), with C forming the electrode surface. The fluidic channels are made of PDMS, and each measure 7 cm long, 5 mm wide and 100 μm deep. These dimensions are physiologically relevant because of the presence of endothelial cells at all dimensions of the arterial system [1, 2], and have been used for the study of NO release from cultured endothelial cells. Inlets at the center and both ends of each channel allow spatially controlled injection of the perfusate (phosphate buffered saline— PBS), the analytes (NO and dopamine) and the introduction of the reference electrode (Ag/AgCl).

3. MULTI-CHANNEL VLSI POTENTIOSTAT

Amperometric sensing over the carbon electrodes requires potentiostatic instrumentation (current measurement at regulated potential). Each potentiostat channel serves one working electrode, maintained at virtual ground, and a redox potential applied to the reference electrode. The potentiostat provides the necessary voltage for driving the redox reactions at the sensor with respect to a reference in the bath and a counter electrode on the electrode array [14].

A two-electrode system can be employed, in contrast with the more traditional three-electrode configuration. This is because the current produced by the oxidation of biochemical species at physiological concentrations is typically in the nano/pico ampere level, and as such, these current levels do not affect the reference electrode.

Wiring connecting sensors to potentiostats can be extensive and noise problems typically arise. Additionally, an array of external commercial bench-top potentiostat instruments, can be prohibitively bulky and expensive. An integrated potentiostat array allows to reduce form factor and minimize interconnect, and to perform data acquisition in close proximity to the sensor array.

Integrated potentiostats with one or few parallel channels have been previously reported by others [10, 11] and our group [12, 13].

3.1. Architecture

The track-and-hold potentiostat [4] in the present system integrates 16 current-mode inputs, 4 voltage references setting the voltage levels of the virtual-ground current inputs in groups of 4, and a single full-range differential voltage output, on a single chip. The block diagram of the single-chip potentiostat is given in Figure 2. Each of the 16 channels is independently configured for a gain of attenuation covering four orders of magnitude allowing to acquire bidirectional currents in the range from 100 pA to 50 μA, at a reference voltage ranging from 0 to 5V. Programmable cut-off frequencies ranging from 50Hz to 400kHz prevent aliasing of high frequency components and allow to decrease the level of noise generated prior to sampling. The maximum fully sustained sampling rate ranges from DC to 200kHz. The outputs of the chip are pipelined and continuously valid, interfacing asynchronously to an external ADC on the PC host acquisition board for data post-processing.

3.2. VLSI Implementation

The input current to each data channel in Figure 2 is summed with a reference current I_{ref}, to convert the signal from bipolar to unipolar form. A transconductance amplifier drives a PMOS load transistor to provide a low impedance input stage. The acquired input current is then fed into a scaling circuit which normalizes the signal to the range [0, 1] μA. The same scaling factor is used to attenuate the reference current I_{ref} at the input stage. The normalized current is fed into an anti-aliasing low-pass filter.

The integrator at the end of the channel is used for current-to-voltage conversion. The timing of integration in one of the reference channels (bottom of Figure 2), supplied a constant current equal to the normalized current range (1 μA), sets the voltage range of conversion in the 16 channels. A 16-to-1 multiplexer selects the integrated signal of one of the 16 channels at the output. The output of the second reference channel, supplied half the current of the timing reference channel, serves as a 'zero-level' reference to the other channels in a differential output format, for reduced sensitivity to noise and power supply variations. In hold mode, the differential output from the previous integration cycle is buffered and held at the output while the current integration process is taking place.

The track-and-hold potentiostat was integrated on a 2.25×2.25 mm^2 die fabricated in a 1.2 μm double-poly CMOS process. The chip micrograph is shown in Figure 3. Input sensitivity is 50 pA in the smallest ($\pm$50 nA) scale, and 25 nA in the largest ($\pm$50 μA) scale. Power dissipation is 12 mW at 5 V supply voltage. Details on the circuits and characterization of the chip are presented in [4].

4. EXPERIMENTS

4.1. Single-Channel Dopamine Recording

The response of the integrated potentiostat and sensor system was calibrated with standard solutions of dopamine as well as NO. In the first experiments a single channel of the potentiostat was interfaced with a carbon micro-fiber electrode to monitor temporal variations in dopamine concentration in a solution, and compare its performance with that of a commercial potentiostat.

A standard solution of dopamine was prepared by dissolving 20mg of dopamine hydrochloride in 99 *ml* of degassed, deionized water. 1 *ml* of perchloric acid was added to keep the solution stable over time. Commercially available carbon micro-fiber electrodes (World Precision Instruments, FL) were utilized. The OD of the carbon micro-fiber was 30 μm. The sensors were also coated with

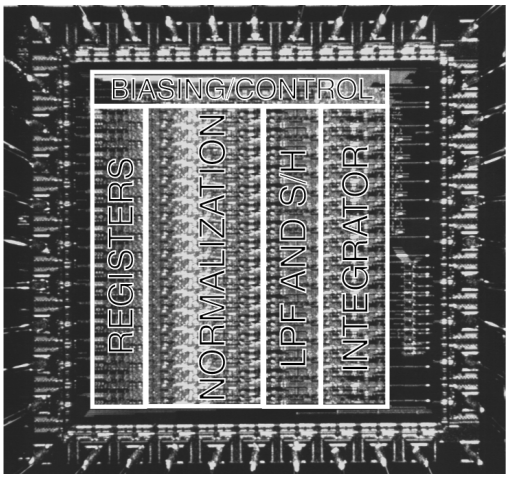

Fig. 3. *Chip micrograph of the 16-channel integrated track-and-hold potentiostat [4].*

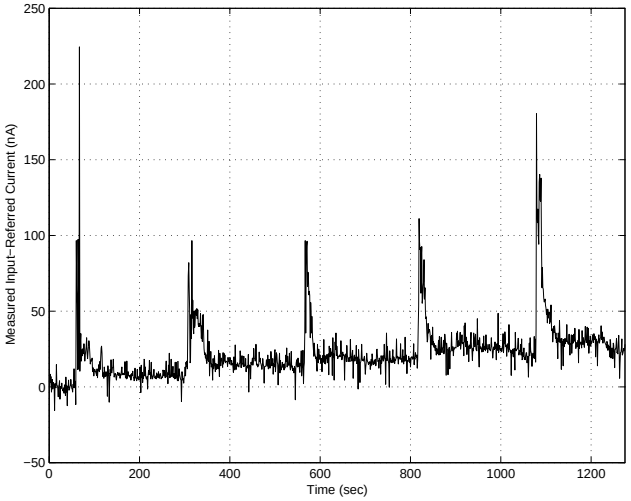

Fig. 4. *Measured input-referred chronoamperometry sensor output current. Controlled amounts of dopamine solution were introduced into degassed phosphate-buffered saline solution every four minutes and briefly stirred.*

Nafion, which is a cationic exchange resin. Its charge selective properties prevent the diffusion of negatively charged ionic species across it, thus preventing such species from reaching the surface of the carbon electrode. However, its presence does not interfere with the oxidation of dopamine. The electrodes were electrochemically activated by successive cycling from 0 V up to +3.0 V for 20 s, +2.4 V for 15 sec and +1.8 V for 10 sec at 70 Hz [15]. This pretreatment increases the sensitivity and stability of the response of the electrodes through the production of an oxide layer and a net increase of surface area.

Each test was conducted in 25 *ml* of degassed phosphate-buffered saline solution (PBS w/o Mg++ and Ca++) at a pH of 7.4. Controlled amounts of dopamine solution were introduced from gas-tight syringes directly into the solution following which the solution was briefly stirred to ensure uniform dissolution of the analyte. The current was allowed to stabilize for four minutes before the next bolus of dopamine was added. Standard chronoamperometry was employed to measure the current, in which the working carbon electrode was held at 900mV with respect to the Ag/AgCl reference electrode. The current was recorded as a function of time as shown in Figure 4. The current measured by the potentiostat registers an initial transient in response to each dopamine bolus addition, followed by a steady rise in the background concentration.

For comparison with the 16-channel VLSI potentiostat, the carbon fiber electrodes were also tested using a commercial benchtop potentiostat/galvanostat (EG&G Princeton Applied Research, Model 273, Princeton, NJ) controlled by a microcomputer via a GPIB interface. The comparative performance is depicted in Figure 5.

4.2. Multi-Channel Spatial Sensing of NO in Fluidic Channel

A second set of experiments interfaced four potentiostatic channels with four of the carbon electrodes along the fluidic channel, as depicted in Figure 1. Figure 6 records redox currents from the four sensors, monitoring diffusion of NO into the fluidic channel, as it is injected on one side.

In Figure 6 (a), 50 μl of standard NO solution was added and the redox currents from the electrodes held at +900mV were recorded. The working carbon electrodes were held and +900mV with respect to an Ag/AgCl reference electrode. Each channel was sampled at 200ms time interval. The temporal difference between the four sensors can be seen. The most upstream sensor (#1) responded first, while the response of the sensors further downstream (#2 through #4) were slightly and gradually delayed. The analyte was then washed out with PBS, and the sensors returned to baseline current levels.

The response of the fluidic channel to injection of a smaller amount (10 μl) of standard 2mM NO solution was added to the channel, which already was full with PBS. As before, the most upstream sensor #1 responded to the NO bolus first, followed immediately by sensor #2. At this level of concentration, the further downstream sensors #3 and #4 did not appreciably respond, indicating that NO did not sufficiently diffuse downstream. Again PBS was used to flush out the fluid after the injection of NO.

Because of the electrochemical technique being used to sense the concentration of NO, the sensors are inherently susceptible to the presence of interfering electroactive species. It has been shown by our group [3] that with the use of surface modifying polymer layers, a selective response to NO can be achieved without sacrificing the sensitivity of the technique. In the present form, the uncoated sensors could detect spurious signals from interferents.

5. CONCLUSIONS

A multi-electrode NO sensor array was interfaced with a multi-channel potentiostat to sense spatiotemporal dynamics of biochemical diffusion and transport in microfluidic channels. In comparison with previously reported NO sensors, the present system integrates a large number of NO electrodes, interfacing with microfluidics and potentiostats, in a single unit for simultaneous multi-channel NO measurement. The performance of a single channel of the VLSI potentiostat was shown to be comparable to that of a commercially available benchtop instrument, at signifi-

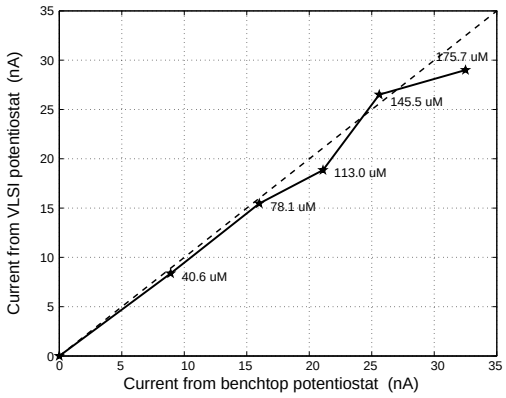

Fig. 5. *Comparison of amperometric response of the VLSI potentiostat with that of a commercially available bench-top instrument, using same carbon electrode, for a range of dopamine concentrations in the solution.*

cant reduction in form factor. Real-time recording of NO diffusion in a laminar channel was experimentally demonstrated. Applications of the integrated sensor include real-time spatial sensing of electrochemical transport in vascular flow, and spatial monitoring of neurotransmitter release.

6. REFERENCES

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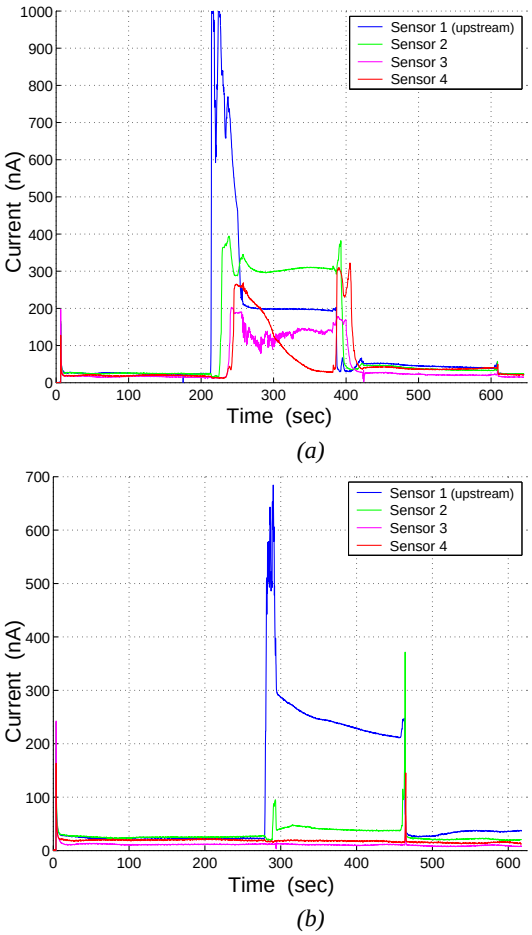

Fig. 6. *Diffusion of NO along fluidic channel, as recorded using four channels of carbon electrode array and potentiostat. (a): 50 μl injected NO. (b): 10 μl injected NO.*

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