Electrochemical Biosensor Array Characterization

Matt Jibson

November 22, 2008

Abstract

Advancing our understanding of how the central nervous system works under specific conditions requires real-time, simultaneous detection of a number of key signaling molecules, like nitric oxide (NO). NO diffuses widely and rapidly, has a lifetime in milliseconds, and presents among other high concentration, interfering compounds in the nanomolar range in most biological systems. Current microelectrode-based electrochemical NO sensors have diameters in the micrometer range, much larger than biological cells which are in the micron range, and are thus insufficient for analyzing cell-to-cell interactions. A chip was fabricated using the $0.6\mu m$ CMOS process to overcome these difficulties, with a 5×5 array of sensors in the $2\mu m$ range. Characterization results of the chip indicate sensitivity into the $20\mu M$ range and suggest higher-sensitivity devices can offer improvement.

1 Introduction

2 Electrochemistry Background

3 Silicon Technology Background

4 Experimental Setup

All experiments were done on a Micromanipulator, Inc. Probestation. Probe tips and other objects (e.g., pipette tips, thermometer) were held in place by micromanipulators. All electrochemistry experiments were done with a CH Instruments 1207 potentiostat.

Fabrication was done at Avago, Inc., which produced two 6-inch wafers. The wafers were diced by Aspen Technologies to separate the chips. To prepare a single chip, a well of PDMS was constructed such that the exterior would not exceed the edge of the chip and would allow for pins to be lowered onto the probe pads, and the interior would allow the sensor array to be exposed (Fig. 1). This was done so that solution and ovaries could be put over the sensor array with no interference to the probe pads. The well was attached to the chip with glue, producing a seal that would not allow leakage of the interior solution to the probe pads. The well was high enough so that the interior could hold at least $70mL$ of solution. The interior was wide enough so that ovaries could be easily attached to the surface.

The first characterization of the chip was needed to find the best electrode cell configuration, i.e., the cell with the largest response for a given input. Recall that there are 25 sensor areas. Each area contains multiple working electrodes, and one or more reference and auxiliary electrodes. Two or more working electrodes were tested at each sensor area. Multiple cyclic voltammetry (CV) runs were performed on each electrode from $0.5V$ to $-0.5V$ at $0.1V/s$ in $1M$ NaCl. The value of an individual run was taken to be the magnitude of the difference of the averages of the two potential sweep directions between $-0.2V$ and $0.2V$. That is, the average value from $0.2V$ to $-0.2V$ in one potential sweep direction was found; the average value from $0.2V$ to $-0.2V$ in the other potential sweep direction was found; the difference of these av-
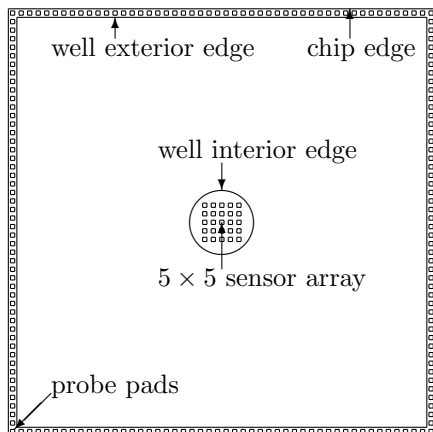
Figure 1: Diagram of chip with PDMS well from above.

erages was found by subtraction. Finally, all results were grouped and averaged per sensor and the sensor area corresponding to the largest result was taken to be the best electrode cell configuration.

To find the ideal potential at which to run the amperometry characterization, a hydrodinamic voltammogram (HDV) was performed to find the optimum potential for amperometry. This was done by running multiple amperometry experiments at identical conditions except for potential, which was varied between $0.6V$ and $1.25V$. In order to ensure identical conditions, the electrode was cleaned before each run by CV from $1.5V$ to $-0.8V$ at $1V/s$ for 100 cycles in H_2SO_4. The chip well was filled with $50\mu L$ $0.1M$ KCl. A syringe of $600\mu M$ norepinefrin (NE) was loaded into a pump. The end of the syringe was connected to a pipette, the end of which was lowered into the well so that it was below the surface of the solution. An ameprometry experiment was carried out where the syringe pump would be enabled for 10 seconds at $100uL/min$. The value of the experiment was taken to be the difference between the idle point just before the syringe pump was enabled (generally close to 0) and the lowest point of the resulting curve. The optimum potential was found by taking the largest result.

The final stage of characterization was done to build a find the current generated by a given con-

centration. This was done using similar conditions as in the HDV characterization. The electrode was cleaned using the CV process with H_2SO_4 as listed above. The well was filled with $50\mu L$ $0.1M$ KCl. A syringe was filled with various concentrations of NE, to which a pipette was connected to the end and lowered into the solution belowe the surface. Amperometry was performed at $0.85V$. The syringe pump was enabled for 10 seconds at $100\mu L/min$ after the potentiostat read a stable value close to zero. The value was taken to be the difference between the idle zero point and the lowest point of the resulting curve. This was repeated for each concentration.

Furthermore, the previous experiment was done using both 2 and 4 working electrodes shorted together in parallel (which was possible due to the common reference and auxiliary electrodes for the sensor area we were using). This created a larger effective surface area on the working electrode, theoretically able to sustain a higher current. To verify that this was a valid technique CVs were performed using $100\mu M$ NE in neurobasal (NB) from $-0.2V$ to $1.0V$ at $0.025V/s$. The value was taken to be the lowest recorded point.

Many ovary slices were tested throughout characterization experiments. These were done by preparing a chip with a well and testing it for basic functionality by performing an arbitrary CV. Ovaries were extracted from mice and attached to the surface of the chip over the sensor array, covered in vitrogen. Neurebasal was periodically added to keep the cell from dying and drying out. The ovaries and chips were kept at $37\,^{\circ}C$ in order to keep them alive. A heat lamp was used during testing with a thermometer measuring temperature directly above the well in an effort to keep the temperature at $37\,^{\circ}C$. Amperometry tests were run at $0.85V$ for 30 minutes. After the main test, three injections were performed, each of $20\mu L$: (1) a blank of NB to test for injection noise, (2) $25\mu M$ NE in NB to test the chip's current response to NE, (3) a 1:1 ratio by volume of KCl:NB to release all remaining NE from the cell, which would provide the maximum expected signal from the main 30 minute block.
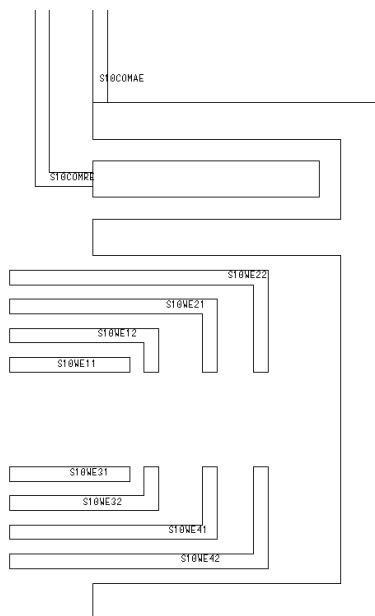
2

Figure 2: Sensor 10 diagram.

5 Experimental Results

Of the 21 different sensor area configurations (numbered 0 - 20), sensor 10 (Fig. 2 provided the highest average results over the $-0.2V$ to $0.2V$ range. This sensor has common auxiliary and reference electrodes and eight individual working electrodes each $8\mu m$ wide. A similarly good sensor (which ranked first under the range $-0.1V$ to $0.1V$) has a similar configuration, but with working electrodes $10\mu m$ wide.

The HDV shows the optimum potential for this electrode measuring NE is $0.85V$ (Fig. 3), which is near the typical value. There is an argument, however, to using a value less than $0.85V$ in order to decrease noise (other chemicals being reduced), which would also decrease the resulting signal. Since we are already near or perhaps at or limit of detection, though, the added noise is acceptable in order to maximize the output.

Concentration response characterization shows reasonable results in the $25\mu M$ to $200\mu M$ range. As expected, the 4-pad set has a sensitivity slightly less than twice that of the 2-pad set. However, both best-
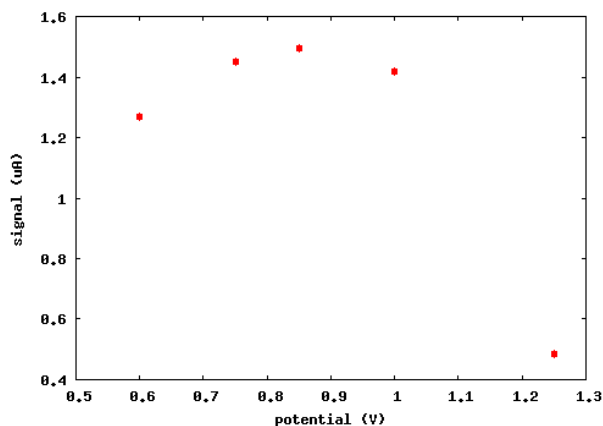

Figure 3: HDV; highest point at $0.85V$.

fit lines do not, as expected, cross the origin, and thus suggest a constant amount of latent noise not dependent on senor area. It is not known whether a potentiostat with improved sensitivity (e.g., into the picoamp range) would be able to reliably detect concentrations below $25\mu M$.

Ovary test results show our potentiostat is not sensitive enough to detect possible NE releases from the cell. The three injections at the end of the testing are not statistically differentiable and do not correspond to our expectations of what should occur. It could also mean the chip sensors were damaged or restricted during ovary attachment and cannot function as expected. {Note: need to have Megan prepare another full chip less ovary and do the three test injections.}
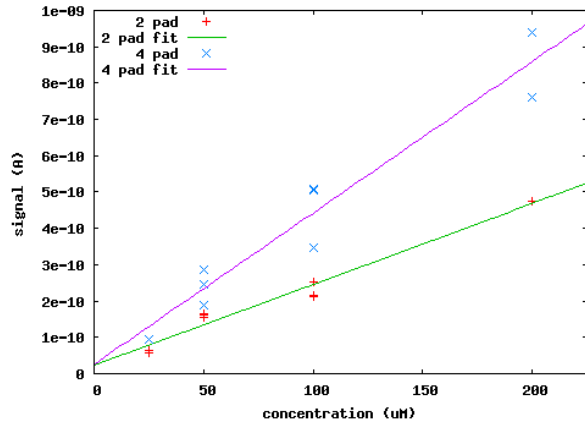
6 Conclusion

Figure 4: 2- and 4-pad characterizations with 1st order best fit lines.