Microfluidic Bio-Sensing for *in vitro* Tumor Cell Proliferation

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Abstract:

We present the fabrication of a monitoring system that allows for monitoring of cells inside an incubator. The system consists of a custom cell-monitoring device, a remote gate ion sensitive field effect transistor (ISFET), an amplifier, and a BeagleBone platform. The design, fabrication, and operation of the remote gate ISFET are described. We also describe how the ISFET was used to create the autonomous, continuous-time cell monitoring system, programmed for any time interval. Finally, we present the results of monitoring cells dosed with the chemotherapy drug *Staurosporine* over a period of 38 hours.

Introduction:

Currently, chemotherapy drug testing on cultured cells is a time-consuming, tedious process. Varying doses of the drug are added to cultured cells, and cells are monitored for viability (percent of the culture flask covered in cells). To accomplish this, researchers remove cells from an incubator (causing their local temperature, humidity, and pH to drop), use a microscope to examine a small area of the flask visually (prone to error from non-uniform distribution), and return the cells to the incubator. Moreover, this long process is just one small step for the diagnosis and treatment of a cancer patient. The ability to decrease the time needed, improve the accuracy of testing, and cater to each individual patient's case is crucial to improving the chances of their survival.

ISFETs are an ideal biosensor for diagnostic testing due to their ability to accurately measure changes in a solution's acidity, such as a cancer cell's microenvironment. Cancer cells have an increased metabolism relative to healthy cells, leading to an increase in the production of lactic acid [1]. We can monitor this increase using the ISFETs as an increase in the pH of the cell culture media.

The aim of this project was to build a continuous-time, autonomous cell monitoring system, for use inside an incubator, with the ability to monitor multiple cell populations for any interval or sampling rate. The reliability of this system was to be confirmed by visually examining the cells over different intervals.

Biosensor Fabrication:

The design for the ISFET biosensor was nearly identical to the design prepared by Welch [2]. However, instead of silicon, quartz was chosen as the substrate to allow for visualization of the cells under a microscope. The pattern for the bio-sensor is shown in Figure 1, with the ion-sensitive portion covered completely in silicon nitride (SiN), and the reference electrode being exposed gold (Au).

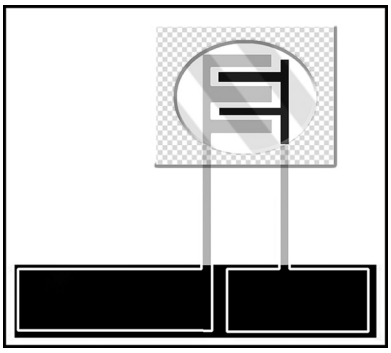

Figure 1: The areas depicted in gray are portions covered by SiN, the black areas are the exposed Au, and the checkered pattern represents the PDMS well.

The fabrication was as follows: photolithography for resist patterning, thermal evaporation for the deposition of 30 nm of chrome and 70 nm of gold, acetone lift-off with sonication, deposition of 65 nm plasma-enhanced chemical vapor deposition (PECVD) nitride, resist patterning, reactive ion etching (RIE) etching, and finally, resist removal. The substrate was then bonded to a polydimethylsiloxane (PDMS) well through plasma cleaning.

Cell Treatment:

About 125 μL of cell media containing breast cancer cells, SKBR-3, was pipetted into the PDMS well. The experimental group was treated with 5 μL of 1 mM *Staurosporine*, a drug known to induce apoptosis in SKBR-3 cells. A control group, not treated with any drugs, was also monitored.

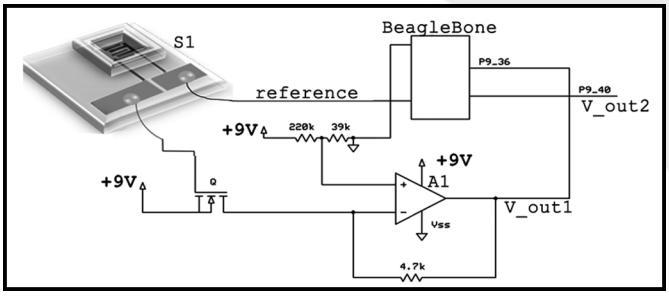

Figure 2: Schematic of the data acquisition system.

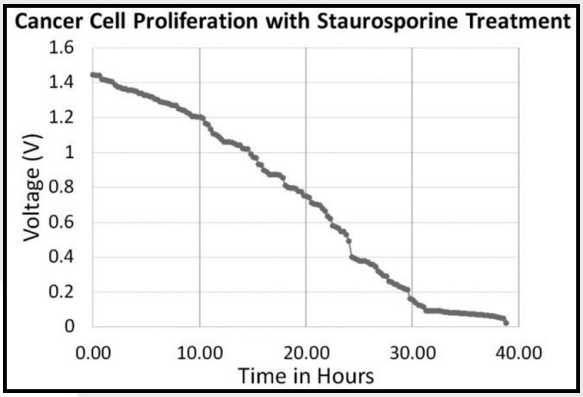

Figure 3: Voltage data collected over a period of 38 hours.

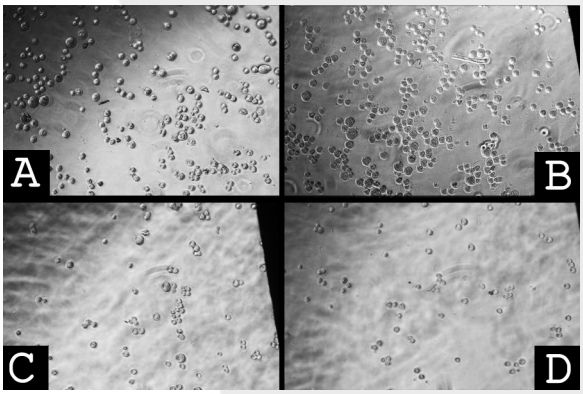

Figure 4: Imaging of the cells at (A) the initial time, (B) after 4 hours, (C) 24 hours, and (D) 36 hours.

Data Acquisition System:

The biosensor's sensing region was connected to the gate of an n-channel MOSFET; while the reference electrode was connected to a BeagleBone platform's (BBP) ground. The output current of the complete ISFET was converted to a voltage by a trans-impedance amplifier before being sent to the BBP for data storage. The complete schematic for each channel is shown in Figure 2. The system, one channel each for the control and experimental group, was powered by a 9V battery, while the BeagleBone was powered by a 5V power supply. The BBP recorded the voltage of the systems in an interval of ten minutes.

Results and Conclusion:

In the first hour of cell-monitoring, the BBP crashed, causing loss of ability to monitor the control group with the data monitoring system. Furthermore, after the fourth hour, the control group evaporated due to the small amount of media the well could hold. The evaporation caused the cells to die, meaning that the control group could not be an accurate comparison to the experimental group. Imaging and data collection from the experimental group continued normally.

The results collected from the BBP are shown in Figure 3. Over a period of 38 hours, the voltage decreased from about 1.4V to 0.02V. The decrease in voltage over time shows that there was a reducing amount of cell proliferation. This agreed with the imaging of the cells shown in Figure 4, confirming that the cell-monitoring system successfully monitored the pH levels of the cells' microenvironment. However, further data will be collected to include a control group.

Further work includes optimization of the BBP to include safeguards for program failure and real-time data acquisition with visualizations over a network, and fabrication of a 2×2 sensor array on one quartz substrate for easier, cheaper monitoring of different cell populations.

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References:

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[2] D. Welch, "Systems Integration for Biosensing: Design, Fabrication, and Packaging of Microelectronics, Sensors, and Microfluidics", Ph.D. dissertation, Dept. Elect. Eng., ASU, Tempe, AZ, 2012.