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A WIRELESS-COMPATIBLE CMOS-BASED CELLULAR SENSOR

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ABSTRACT

Biosensors have a need to accurately detect and quantify a biological target within a complex medium, such as serum. Cells are uniquely qualified to accomplish this, but cannot communicate with the outside world. We demonstrate a synergistic fusion between a genetically engineered cell line and a wireless-compatible CMOS sensor leveraging the cells innate ability to specifically bind, amplify and transduce the signal with electronics that are capable transforming this cellular signal into an electronic one. We demonstrate the detection of TNF-alpha (TNF α) within serum without washing. Since cells are real-time transducers of binding events, real-time monitoring of biological agents and reactions are possible.

KEYWORDS

CMOS, Imaging, Array, cell-based, biosensor, luciferase

INTRODUCTION

Current methods of biological detection rely on binding of the target to highly specific molecules, such as antibodies. This binding event is then transduced through a variety of methods, typically utilizing a labeled secondary antibody. Advances in microelectronic and MEMS-based approaches have leveraged this principle by using charge-based and magnetic-based approaches[1], allowing a direct interface to the sensor. In a clinical setting, the target is contained in a biologically complex fluid, such as serum. This necessitates removing the unbound sample, and multiple wash steps to prevent non-specific binding of the secondary antibody. These requirements necessitate labor intensive washing steps requiring trained technicians, limiting use to centralized facilities or requiring implementation of complex microfluidics. Furthermore washing off the sample prevents real-time monitoring of the sample.

BACKGROUND

In living biological systems, specific ligand detection within a complex medium is continually necessary, but complex washing steps cannot be performed. Cells accomplish this by having highly-specific receptors, as well as internal "signal processing" allowing thresholding and amplification of the binding event. We leverage these properties by using cells to detect the ligand, and have them communicate with a

microfabricated sensor. Optically sensitive biological proteins have been widely used in biology, and span two main categories: fluorescent (i.e. GFP) and luminescent (i.e. luciferase). These have the advantage of being able to be genetically encoded into the cells DNA, and can signal a cellular event when they are synthesized. We use firefly luciferase, as the luminescent properties only require a substrate (luciferin, ATP and magnesium), and has no background.

The difficulty with using luciferase is the extremely low-light levels produced. Although PMTs (photomultiplier tubes) and CCDs (charge coupled devices) have traditionally been employed in the detection of luciferase, Complementary Metal Oxide Semiconductor (CMOS)-based platforms enable versatility, making them ideal for implementing a biosensor. In the case of a cell-based sensor, by integrating the cells directly on (or extremely close to) the sensor, light-gathering optics can be eliminated, reducing both the size and cost of the sensor. Additionally, the use of luminescence, as opposed to fluorescence, obviates the need for optical filters. Both of these techniques enable the sensor itself to be limited only by the size of the CMOS device. This results in a biosensor that can be fabricated at a size appropriate for integration into a biological or *in-vivo* environment.

In order to fully integrate the sensor with the cell, the sensor must be compatible with a fluidic, biological, or *in-vivo* environment. These requirements necessitate a wireless-compatible optical sensor, capable of receiving power and transmitting data wirelessly, while detecting extremely low amounts of light. CMOS provides the optimal platform, due to its ability to integrate optical sensors directly with electronics. Although CMOS sensors have been previously developed to sense luciferase [3,4], no wireless array-based imager exists that is fabricated in standard CMOS process. Here we use a sensor platform developed in our laboratory[2] (Figure 1) to monitor the detection of a ligand by cells genetically engineered to express luciferase upon receptor binding.

SENSOR DESCRIPTION

The sensor is a CMOS-based 64 pixel imager fabricated in a standard 0.18 μm digital process, powered by a single-sinusoidal power supply, and capable of transmitting data wirelessly enabling wireless-

compatibility. Wireless power transfer can be achieved by bonding an external inductor to the imager. The primary difficulty with CMOS-based imagers for low-intensity imaging is the large dark current, typically 3 to 4 orders of magnitude larger than the luminescent signal. The circuit overcomes this limitation by having a fully differential design, with each $125\ \mu\text{m} \times 125\ \mu\text{m}$ photodiode having an identical reference photodiode covered with metal. Ideally, the dark currents would precisely cancel each other, but mismatch between photodiodes results in a differential dark current. Since this current is constant, it is used to calibrate the chip, and can be subtracted from output. The output of each pixel is then amplified and converted to a digital representation by on-chip signal processing electronics. The results can either be wirelessly transmitted off-chip using an on-chip inductor made of several metal layers, or via standard bond-wires.

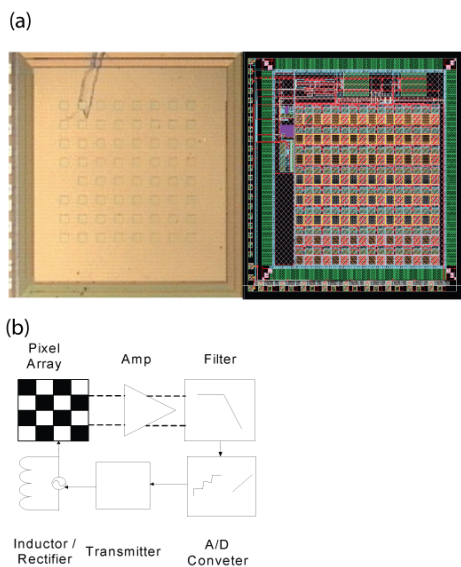


Figure 1: (a) Die photo of the $4 \times 4\ \text{mm}$ chip with 64 pixels. The layout of the chip is shown to the right. (b) Schematic of circuit operation. Each of the 64 pixels is sequentially read out by the on-chip amplifier and converted into a digital representation by a single slope analog to digital converter. The data can be output via standard IO pads, or wirelessly transmitted using the on-chip inductor. The chip is powered by a single sinusoidal power supply, making wireless operation possible with the addition of an external inductor.

We use HEK 293T cells genetically engineered to express firefly luciferase in response to TNF- α (Figure 2). Cells are placed with a solution of 10% Fetal Bovine Serum (FBS), with 50 ng/ml of TNF- α and the expression of Luciferase is monitored via the addition of luciferin. No washing was done. The results are compared with a PMT-based imager in which the cells were lysed, mixed with luciferin, and their contents placed within a

luminometer.

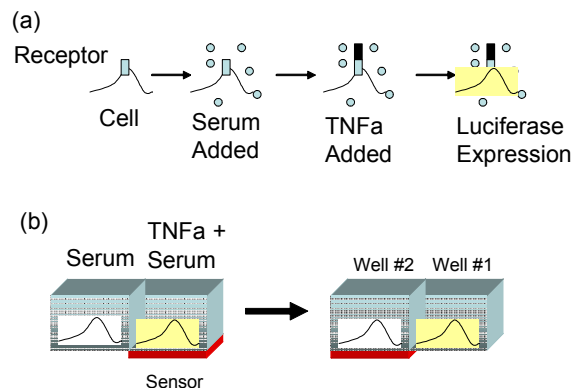


Figure 2: (a) The cell is genetically engineered with a receptor specific for TNF α that links to a gene that produces Luciferase when the receptor is activated. A solution of Luciferin, DTT, ATP and Mg is added to the cellular solution to provide the proper substrate for the luminescent reaction. (b) The cells in the negative control well are exposed to serum, and the cells in the positive control well are exposed to serum and TNF α at 50 ng/ml. The sensor reads one well, and is then moved to the adjacent well.

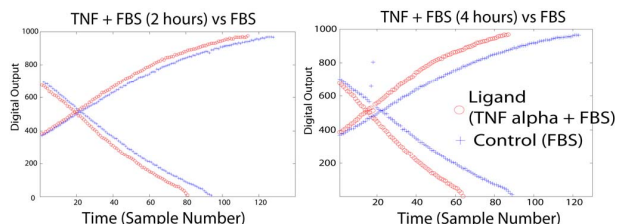


Figure 3: The differential sensor output at 2 hour and 4 hour incubations (Sample number = 87 msec). A more rapid integration time with the positive controls can be seen at both 2 and 4 hours, indicating Luciferase expression and increased light detected by the sensor

EXPERIMENTAL SETUP

To image the cells, the sensor is placed under a coverglass coated with cells, with one well incubated in TNF α plus serum, and the other a control well. The sensor can be moved to image either well (Figure 2b). The cells are placed in an incubator with 5% CO $_2$, and grown for variable amounts of time. Figure 3 shows the integrating trace for a single pixel imaging cells incubated in TNF α plus serum versus serum alone, for incubation times of 2 and 4 hours respectively. The integration trace with TNF α rises faster than the control trace (which is largely due to the differential dark current). After four hours, the control sample is unchanged, but the sample with TNF α shows increased luminescence.

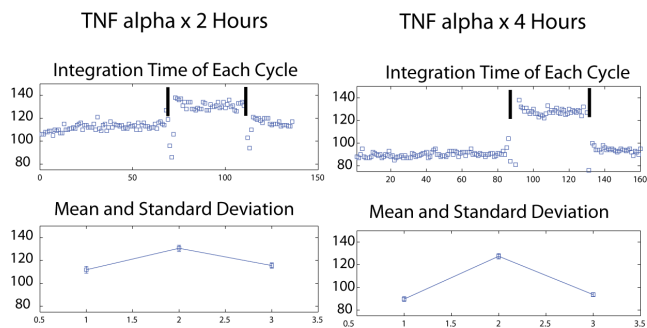


Figure 4: The integration time of each cycle is shown, with the hash-marks representing the transition from positive control well to negative control well, and back to the positive control well.

RESULTS

Figure 4 shows a plot of the integration time, as the sensor is moved from imaging the positive control to the negative control, and then back to the positive control. The lower integration times indicate a greater amount of light detected, corresponding to increased luciferase production. This experiment is repeated at multiple time points, shown in Figure 5. As a reference, an identical set of cells are lysed, and the lysate is mixed with luciferase and imaged with a PMT-based luminometer. The results show a proportional increase in luciferase production with increased exposure time, with a maximal effect at approximately 800 minutes. This corresponds to the cells continuing to produce luciferase until the internal concentration reaches a steady state, balanced by protein destruction, cell division, and down-regulation of protein production.

DISCUSSION

These results show that genetically-engineered cells can be coupled with microelectronic devices to create sensors that can detect specific ligands within a complex mixture without the use of sample processing. This enables simplification of device design as well as the potential for real-time monitoring of ligand concentration. The cells have no background signal in the presence of serum, yet strongly express luciferase in the presence of TNF α demonstrating receptor activation. The expression as a function of time, corresponding to the real-time concentration of the ligand, can be monitored. Furthermore, the wireless interface enables compatibility with implantable applications where cells could be used to monitor physiologic, or pathological markers in real time.

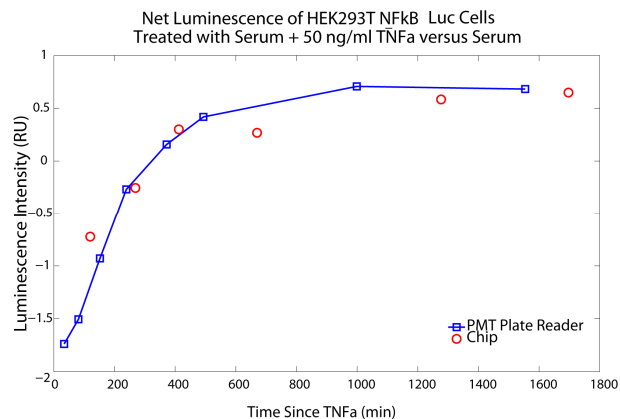


Figure 5: Cells with varying exposure time to TNF α (squares with line) compared to cellular luminescence measured by a luminometer (circles). The luminescence detected by the sensor is proportional to the luminescence detected by the photomultiplier tube, enabling high sensitivity, quantification, and real-time monitoring of analyte concentration.

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