
Somashekar B. Prakash, Nicole M. Nelson, Alfred M. Haas, Victor Jeng, Pamela Abshire
Department of Electrical and Computer Engineering
University of Maryland
College Park, Maryland 20742

Abstract—Cell clinics, CMOS/MEMS hybrid microsystems for capturing and in-situ investigation of living cells, aims at providing high-speed, automated, and economical cell monitoring. Integrated sensors are being developed for extracellular signal amplification, cell-substrate capacitance sensing, contact imaging, and fluorescence detection. We describe the methodology for characterizing the responses of these sensors to biological cells. We also present results obtained from the long-term monitoring of cells cultured on-chip using two of the sensors: (i) a bioamplifier, used for amplifying weak extracellular potentials from electrically active cells, and (ii) a cell-substrate capacitance sensor, used for tracking cell adhesion and assessing cell viability.

I. INTRODUCTION

In order to gain a deeper understanding of the operation of biological cells, and to learn how to exploit their sensitivity to environmental parameters for sensing applications, we have developed integrated CMOS sensors to measure their in vitro behavior and response to stimuli. More specifically, we have developed CMOS sensor arrays to amplify extracellular potentials [1], monitor cellular capacitance [2], and image the positions of biological cells [3]. The measurements made using these sensors have shown strong correlations with depolarization events, cell viability, and location, respectively. Integrating these sensors into a CMOS/MEMS microsystem, our measurement suite, or “cell clinic” [1], aims to perform measurements rivaling those of conventional instrumentation, but operating at power levels, size, and cost that are orders of magnitude smaller.

II. CELLS ON CHIPS – HOW IS IT BEING DONE?

The problem of packaging integrated biosensors is an obvious one: how to keep the electrical leads dry and insulated, while exposing only the sensors (just tens of microns away), to the aqueous cellular environment. This often overlooked problem can be a “show stopper.” One can conceive ways of solving this problem, but small tolerances, multiple length scales (from μm to cm), biocompatibility, and electrical requirements are some of the core challenges involved. We describe our approach for tackling the above mentioned challenges, which allows us to characterize sensor responses to cells cultured on-chip.

A. Chip Fabrication and Packaging

The sensors were fabricated in a commercially available 0.5 μm, 2-poly 3-metal CMOS process. The sensor chip was packaged in a standard 40-pin DIP ceramic package. If the sensing electrodes (fabricated using aluminum) are required to be exposed to the electrolyte for direct contact with cells, they are electrolessly gold plated to make the surface biocompatible and electrochemically corrosion resistant. The chip is then encapsulated using Loctite 3340, a photopatternable (365 nm) and biocompatible polymer. The patterning process is quick (on the order of minutes) and has been described elsewhere [4]. The chips are encapsulated using one or two polymer levels, depending upon the access requirements. Fig. 1 shows photographs of a 3×3 mm² CMOS/MEMS chip before and after Loctite encapsulation. A well for containing the cell culture is then glued over the encapsulation [2].

B. Sensor Testing with Cells Cultured On-Chip

All the sensing experiments were conducted with bovine aortic smooth muscle cells (BAOSMCs). Fig. 2 shows BAOSMCs adhered to an on-chip electrode. These cells exhibit spontaneous electrical activity that depends on their state...
of health or age. BAOSMC loading into the sensor well is performed under standard aseptic conditions. The chip is then mounted onto a test board and placed inside a Faraday cage for noise immunity. The assembled test fixture is maintained under standard aseptic conditions. The chip is then performed under these excitation conditions [2]. Cell-substrate capacitances to measured voltages. Cell monitoring experiments with BAOSMCs have demonstrated that on-chip capacitance measurements can track the cell-substrate interaction process and respond to changes in cell viability [2]. Fig. 4 shows a four day plot of the sensed capacitance as recorded by a sensor with a sensing electrode area of 40×40 µm^2. The cells were continuously monitored in a closed, undisturbed environment on top of the sensor chip, without growth medium replenishment. As shown in the figure, the capacitance tracks the initial sedimentation and adhesion phases over the first few hours. Then the capacitance exhibits many fluctuations, indicating ongoing cell activity. Over the last two days the time averaged value of the measured capacitance levels out, indicating compromised viability and inactivity due to starvation and lack of oxygen.

V. Future Work
Validation experiments employing traditional cell biology techniques are currently in progress for characterizing these sensors. Extracellular potentials acquired from the bioamplifier will be compared with those obtained from standard electrophysiology techniques such as “patch clamp.” Capacitance sensor responses to cell adhesion and viability will be correlated with spectrophotometric measurements using standard cell viability dyes such as Alamar blue and MTT assays. In addition to the above, other on-chip cell sensing techniques, such as contact imaging and fluorescence detection, are also being investigated and developed.

REFERENCES