

A CMOS Capacitance Sensor That Monitors Cell Viability

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Abstract—We describe a CMOS capacitance sensor for measuring the capacitive behavior of living cells in a culture environment, in the presence of weak electric fields. The underlying physical phenomenon results primarily from polarization of the ionic cloud surrounding the cell in aqueous medium. The measured capacitance depends on a variety of factors including cell morphology, membrane integrity, medium pH and extra cellular ionic concentration and serves as an indicator of cell health. The capacitance sensor uses the principle of charge sharing and maps sensed capacitance values to voltages. The sensor chip has been fabricated in a commercially available 0.5 μm , 2-poly 3-metal CMOS technology. The sensors have been successfully used for long term monitoring of cell viability *in vitro*.

I. INTRODUCTION

Interfacing integrated microelectronic sensors with biological cells has immense potential for numerous applications in healthcare, defence and bioengineering [1]. Electronic biosensing techniques are noninvasive in that they monitor the responses of living cells in real time without altering the biochemical composition of the extracellular environment. This prevents unnecessary modification of the *in vitro* cellular environment which can interfere with the analysis procedure and produce unintended side effects. An integrated system-on-a-chip offers versatile solutions to complex biosensing problems by automating the sensing and analysis procedures, thereby reducing the infrastructure and cost requirements and also enabling measurements outside the confines of a cell biology lab.

The electrical properties of biological cells have a strong correlation with their morphological and metabolic states [2]. For example, the existence of the membrane potential is a distinguishing feature between living and non-living cells. Impedance measurements of cells over different frequency ranges can differentiate between normal and abnormal cell types.

We have developed a CMOS biosensor for monitoring cell viability and surface adhesion by sensing the capacitive coupling between a sensing electrode and the cellular matrix. The sensing electrode in our case is electrically and biochemically isolated from the cell environment. When exposed to low frequency, low strength electric fields, living cells in growth medium behave as insulating structures surrounded by ionic clouds that compensate fixed charges present in the membranes

[3]. An electric field polarizes the ionic cloud giving rise to electric dipoles which are the dominant factor responsible for the low frequency capacitive behavior of cells. Healthy cells with well formed plasma membranes sustain stronger electric dipoles than dead or unhealthy cells with compromised membrane structures, so the measured capacitance is higher for healthy cells. In addition, healthy cells adhere more tightly to a surface in comparison with unhealthy or dead cells, which results in stronger capacitive coupling between the cells and underlying electrodes. Both of these properties can be exploited to monitor the morphology of cells as well as their interaction with substrates.

II. CELL-SUBSTRATE INTERACTION

Cell-substrate interaction plays a crucial role in the lifecycle of a majority of cell types. This is because most normal living cells need to be attached to a substrate before they can grow and proliferate. In addition to its biological significance, understanding cell adhesion has many practical applications in the fields of medicine, bioengineering and environmental sciences. For example, the formation of biofilms (complex aggregations of microorganisms on solid surfaces) is important in a variety of applications in food and water quality assessment and treatment [4].

A. Biophysical origin of cell-substrate capacitance

In the presence of low frequency, low strength electric field excitations, living cells behave as insulating structures suspended in an electrically conductive growth medium which is an aqueous ionic solution. In addition, cell surfaces generally carry a surface charge density, which can be positive or negative depending upon the cell type [3]. The majority of cell surfaces are negatively charged. This induces a counterionic cloud around the cells in the surrounding medium. When exposed to an external electric field these counterions move tangentially around the cell surface giving rise to an induced dipole moment as illustrated in Fig. 1. Both the insulating nature of cells at low excitation frequencies and the counterionic polarization are responsible for the capacitive behavior of cells.

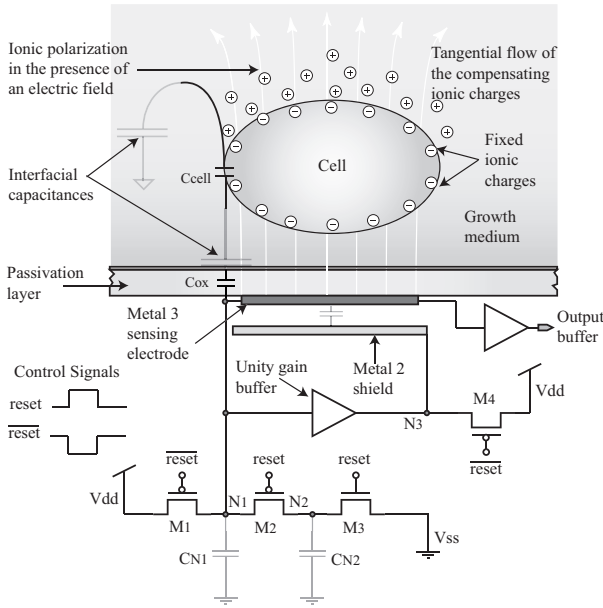


Fig. 1. Cell-substrate capacitance sensor: design and operation.

B. Correlating cell-substrate capacitance with cell-substrate interaction

Next we examine the behavior of a cell culture suspension when it makes contact with a solid biocompatible substrate. Proteins in the growth medium spontaneously adsorb onto the substrate. The interaction between cells and substrate starts off with the sedimentation phase when the suspended cells gradually drift downwards and settle on the surface. This is followed by the adhesion phase when the cells anchor themselves to the surface through various mechanisms both at the molecular and cellular levels [5]. This is also accompanied by a significant change in cell morphology wherein the cells exhibit a spreading behavior. Finally, under favorable conditions there is a proliferation phase during which cells divide and proliferate. In the presence of weak, low frequency electric fields all three phases can be modeled as a process of cell dielectric layer formation as shown in Fig. 2.

The capacitance arising from this dielectric layer successively increases in the phases described above. The cell-substrate capacitance is lowest in the sedimentation phase since the dielectric layer is not yet completely formed. During the adhesion phase there is a remarkable decrease in the dielectric layer thickness due to cell spreading and anchoring mechanisms. In addition, the effective dielectric constant of the cell layer increases due to increasing cell membrane surface area and increasing cell dipole density. Both factors contribute to a steady increase in the cell dielectric layer capacitance. Once the cells have adhered to the surface and adjusted to the culture conditions, the proliferation phase begins and the cell layer capacitance is expected to fluctuate since the dielectric layer is in a continuous process of reformation due to cell activity. In cases of adverse conditions, the growth phases described above may be superseded by a cell death phase

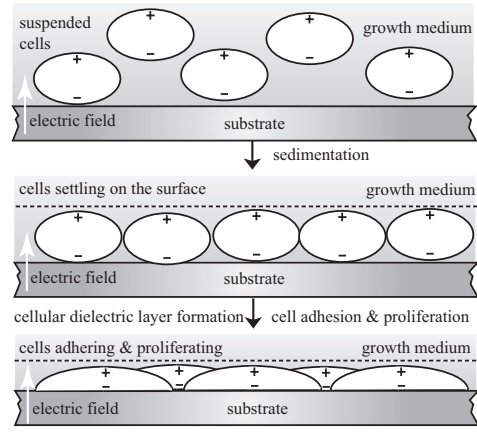


Fig. 2. Cell dielectric layer formation in the presence of weak, low-frequency electric fields: sedimentation phase (top, middle), adhesion and proliferation phases (bottom).

during which the plasma membranes begin to disintegrate, causing the cell dielectric layer to reduce its capacitance.

III. CELL-SUBSTRATE CAPACITANCE SENSING

The purpose of this work is to develop a microelectronic sensor for inexpensive, portable and reproducible characterization of cell viability properties, by quantifying the biophysical phenomena described in the previous section. Towards this goal, a custom CMOS capacitance sensor has been designed using the topology shown in Fig. 1 [6], [7].

A. Sensor design and operation

The sensor operation is based upon the charge sharing principle. The sensor circuit has two nodal parasitic capacitances C_{N1} and C_{N2} whose charging and discharging are controlled by a set of three MOSFET switches M1, M2 and M3. The sensor operates in two phases. In the reset phase, switches M1 and M3 are turned on, charging node N1 to V_{dd} and node N2 to V_{ss} , while M2 is off. In the evaluation phase, M2 is turned on, while M1 and M3 are off, redistributing the charges between C_{N1} and C_{N2} . The joint nodal voltage V_N is a function of the sensed capacitance C_{sensed} as a result of the charge redistribution.

$$V_N = \frac{(C_{N1} + C_{sensed})V_{dd} + C_{N2}V_{ss}}{C_{N1} + C_{N2} + C_{sensed}} \quad (1)$$

Referring back to Fig. 1, the topmost metal3, forms the sensing electrode. Sensitivity of the measurement is maximized by minimizing the nodal parasitics. For this purpose, the fringe capacitances between the sensing electrode and the substrate are shielded by means of a larger area metal2 plate in the lower layer. The large capacitance between the sensing electrode and the shield is cancelled by driving the metal2 shield with a potential that tracks the sensing electrode potential using a unity-gain buffer. Sensor dynamic range improves with increasing sensing electrode area.

The circuit has been designed for a supply voltage of 3 V and has been fabricated in a commercially available

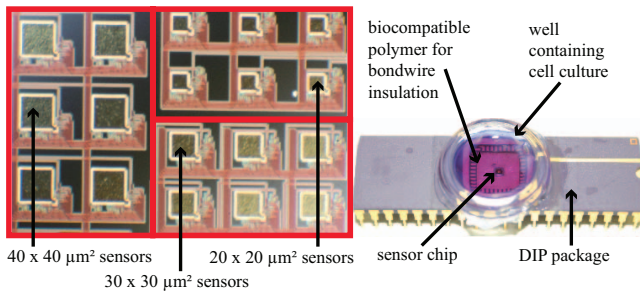


Fig. 3. Left, photomicrograph of the fabricated sensors. Right, photograph of the biocompatibly packaged sensor chip.

0.5 μm CMOS technology with 3 metal layers. Three sensor groups with electrode areas of $20 \times 20 \mu\text{m}^2$, $30 \times 30 \mu\text{m}^2$ and $40 \times 40 \mu\text{m}^2$ have been designed and tested. Fig. 3 shows a photomicrograph of the fabricated sensors. The sensor chip has been used to characterize cell-substrate capacitance at a reset frequency of 1 kHz, as described in further detail in section IV.

B. Sensed capacitance modeling

Several factors influence the capacitance measured at the sensing electrode by the circuit. These factors include:

1) *Passivation layer capacitance*: The passivation layer of the fabrication process isolates the sensing electrode from the cell environment. For a passivation layer with uniform thickness of 1 μm and a dielectric constant of 6, the capacitance per unit area is approximately $0.05 \text{ fF}/\mu\text{m}^2$.

2) *Interfacial capacitances*: The passivation layer (a solid surface) is in direct contact with the cell growth medium (an aqueous ionic solution), resulting in a layered polarized interface according to Gouy-Chapman-Stern theory [8]. This gives rise to interfacial capacitances of the order of $100 \text{ fF}/\mu\text{m}^2$, 3-4 orders of magnitude larger than the passivation layer capacitance.

3) *Cell layer capacitance*: As discussed in section II, after sedimentation the cells form a complex dielectric layer at the growth medium-passivation layer interface. Ionic conductances can be neglected in the model since the cell environment is exposed to weak electric fields with no current flow. Thus the cell layer can be regarded as purely capacitive. In reality, the cells form a heterogeneous layer which exhibits spatial and temporal variation of dielectric properties. Assuming that the dielectric constant of the insulating cell layer depicted in Fig. 2 is equal to that of the plasma membrane = 9.04 [9] and that the cell diameter is 5-10 μm , the cell layer capacitance is on the order of magnitude of $0.01 \text{ fF}/\mu\text{m}^2$, comparable with that of the passivation layer.

From the above discussion and referring back to Fig. 1, the capacitance as seen by the sensing electrode equates to the series combination of the passivation layer, cell layer and all the interfacial capacitances between various liquid-solid boundaries. Comparing the relative order of magnitudes, the effective value of sensed capacitance is dominated by the passivation and cell layer capacitances.

$$\frac{1}{C_{sensed}} = \frac{1}{C_{passivation}} + \frac{1}{C_{cell}} \quad (2)$$

Under equilibrium conditions the bulk of the growth medium is electrically neutral and is free of any potential gradients. This provides a reference potential for the capacitance measurement, although there is no direct electrical connection between the growth medium and the circuit.

C. Sensed capacitance computation

The transducer was calibrated as a proximity detector by using an external metal electrode whose vertical positioning was controlled by means of a piezoelectric micropositioner. Based upon bench test results the nodal parasitic capacitances C_{N1} and C_{N2} were estimated using least squares fits to be 20 fF and 18 fF respectively [7]. In order to translate the sensor outputs to sensed capacitance values, the output voltages during the evaluation phase are subtracted from their corresponding reset voltages for offset cancellation. The inverse relation for C_{sensed} as a function of this voltage difference can be derived from (1) as

$$C_{sensed} = \frac{(V_{dd} - V_{ss})C_{N2} - V_{diff}(C_{N1} + C_{N2})}{V_{diff}} \quad (3)$$

where $V_{diff} = V_{reset} - V_{eval}$ and $V_{reset} = V_{dd}$. Here both V_{reset} and V_{eval} refer to the voltages before the readout buffer.

IV. SENSOR RESPONSE TO LIVING CELLS

For the purpose of *in vitro* testing, the sensor chip in a 40 pin DIP was packaged using a biocompatible polymer for bond wire insulation and isolation of cells from toxic materials of the chip package. A well was formed on top of the chip surface for containing the cell culture. Fig. 3 shows a photograph of the final assembly.

A. Expt 1: Monitoring cell viability

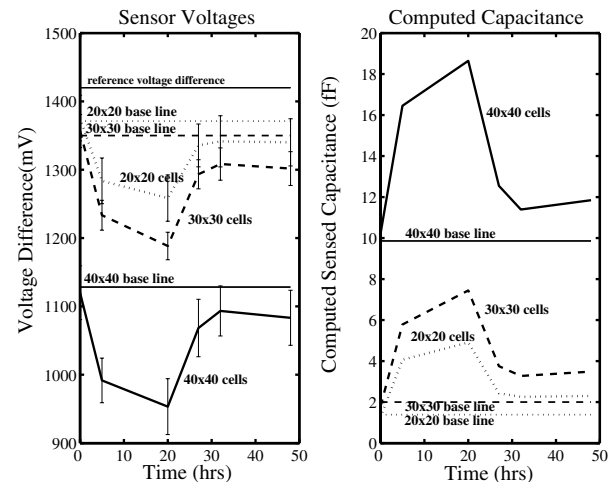


Fig. 4. Averaged sensor response to changes in cell viability.

For this experiment the well was loaded with bovine aortic smooth muscle cells (BAOSMC) stained with neutral red

in a colorless growth medium and the sensor outputs were monitored over a period of 48 hours. In between measurements the fixture was maintained in an incubator at 37°C, 5% CO₂. The sensed capacitances were computed as discussed in section III C. Fig. 4 shows plots of the average voltage differences and measured capacitances for the three sensor groups, with all values aligned to zero sensed capacitance reference. Error bars indicate the standard deviation of the sensor outputs within their respective groups.

Viability was assessed independently through visual inspection of the stained cells. Living healthy cells have the characteristic property of taking up and retaining neutral red whereas non-viable cells do not retain the stain [10]. Over the first day the cells were able to retain the stain and the sensors showed an increase in the cell-substrate capacitance. On the second day it was found that the cells had released the dye into the medium, an indication of non-viability. Accordingly the sensors showed a drop in the sensed capacitance values.

B. Expt 2: Online tracking of cell adhesion process

A data acquisition system was setup for online monitoring of the sensor responses to cells loaded on top of the chip surface and placed inside the incubator. The sensor readings were recorded every 5 minutes with the cells exposed to electric field excitations only during the short recording intervals. Cell viability was confirmed using alamar blue, a viability stain. Alamar blue is available in an oxidized, blue, nonfluorescent form, which becomes gradually reduced to pink fluorescent form in a medium containing viable cells.

BAOSMC loading and incubation were performed in the same manner as in the previous experiment but this time with growth medium containing alamar blue stain in a 1:10 ratio. Fig. 5 shows the sensed capacitances as recorded concurrently by six 40×40 μm² sensors during the first 8 hours of cell incubation. The fraction of alamar blue in reduced form was obtained from spectrophotometric readings of microsamples extracted from the sensor well, at three times during the monitoring period: 0, 3.5 and 8 hours. The capacitance plots clearly illustrate the sedimentation and adhesion phases as discussed in section II. The cells took around 2-4 hours to sediment and around 30 minutes to 1 hour to adhere. The figure also shows phase delays in the initiation of cell adhesion as recorded by sensors in different locations. Alamar blue was gradually reduced to its pink form during the 8 hour interval, confirming cell viability. According to spectrophotometric readings, the fraction of alamar blue in reduced form was 0%, 50% and 84% at 0, 3.5 and 8 hours respectively with reference to the initial cell loading time.

V. CONCLUSION

A CMOS capacitance sensor chip has been designed to measure cell-substrate capacitance, an indicator of cell health and morphology. Biophysical factors contributing to the sensed capacitance have been identified. The sensor chips have been bench tested for capacitance calibration and computation. *In vitro* test results with BAOSMC show that the sensors are able

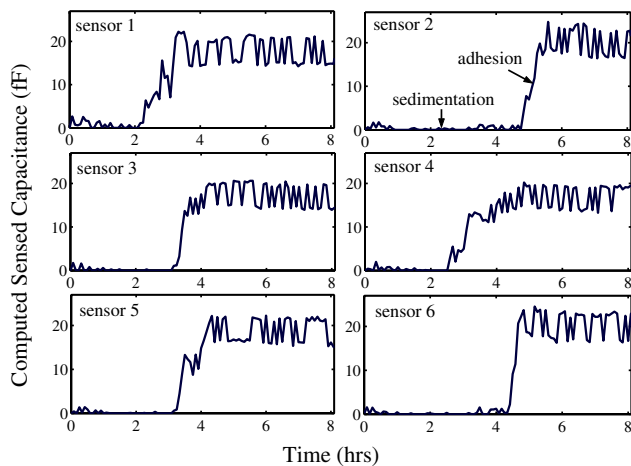


Fig. 5. Online tracking of cell adhesion process.

to detect cell-substrate capacitive variations in the fF range, with different sensing ranges for the three sensor groups. Results from long term monitoring show that the sensors have been effective in tracking cell viability and different phases of the cell-substrate interaction process. This can serve as a useful tool for a variety of cell monitoring applications including biocompatibility characterization, biochemical detection and medical diagnosis.

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