Impedance-based Biosensors

X. Huang,[1] D.W. Greve,[1] I. Nausieda,[1] D. Nguyen,[2] and M.M. Domach[2]
[1]Department of Electrical and Computer Engineering, Carnegie Mellon University, Pittsburgh, PA 15213, U.S.A.
[2]Department of Chemical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213, U.S.A.

ABSTRACT

Impedance measurements on arrays of microelectrodes can provide information about the growth, motility, and physiology of cells growing on the electrodes. In this talk, we report recent results obtained for the growth of 3T3 mouse fibroblasts and HCT116 human cancer cells on gold electrodes approximately 0.4 mm^2 in area. Cells produce a characteristic peak in the impedance change plotted as a function of frequency. With the aid of electrical modeling of the cell-electrode system, the details of the changes in the measured impedance can be correlated to the cell size, fractional electrode coverage, and cell-electrode gap. In particular, comparison of impedance measurements of these two cell types show clear differences in the growth rate and the ratio of the cell-electrode gap to the cell size. In addition to presenting these experimental results illustrating the utility of electrode impedance measurements, we will outline the issues encountered when electrodes are scaled to cell size and incorporated into a matrix-addressed array.

INTRODUCTION

Impedance-based microelectrode sensor arrays are potentially useful for performing drug screening experiments and also for studies of cell adhesion and micromotion. The use of impedance sensors to study cell behavior was first reported by Giaever and his coworkers in 1986 who monitored cell proliferation, morphology, and motility [1]. The impedance sensor consists of two metal electrodes: one large common reference electrode and one small working electrode. When cells are cultured on the electrodes the measured AC impedance changes in a way which depends on the frequency of measurement, the cell coverage, and the cell-electrode gap. We are developing arrays of cell-sensing electrodes which can be used to monitor many individual cells or many individual clusters. In the following, we first briefly outline the impedance changes which result from cell growth and the relation between these impedance changes and the cell coverage and cell-electrode gap. We then present data on the spreading and growth of normal mouse fibroblasts and human cancer cells. Finally, we describe the design of an electrode array fabricated using a CMOS technology and discuss some of the design issues.

Figure 1 shows the basic sensor configuration. A small sensing electrode and a larger counterelectrode are immersed in cell growth medium. Except at very high frequencies, the measured impedance is dominated by the surface impedance of the smaller electrode. That surface impedance depends on transport through the electrical double layer and monotonically decreases in magnitude with increasing frequency. Cells growing on the electrode adhere to the electrode only at focal adhesion regions which represent a small (5-10%) fraction of their area. At low frequencies, current can flow from regions beneath the cell and through the medium in the cell-electrode gap and there is no change in the measured impedance. At moderate frequencies, however, cells obstruct the current flow and there is an increase in the measured impedance. Analytic theory [2] and simulations [3] show that cells lead to a characteristic peak

in the impedance change as a function of frequency. The magnitude of the impedance change depends on the cell coverage and the cell-electrode gap.

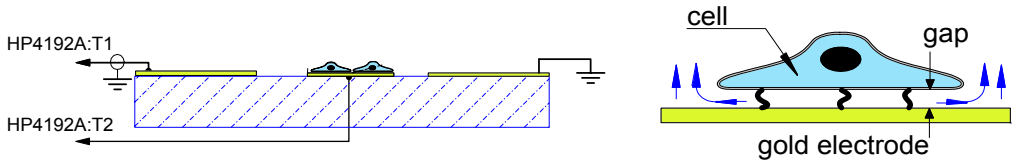

Figure 1. Effect of cell on measured electrode impedance (left) small sensing electrode and large counter-electrode and (right) cell on sensing electrode showing current flow through the gap between the cell and the electrode.

Figure 2a,b show a lumped equivalent circuit which represents the electrode with and without cells. Because of the strong frequency dependence of the electrode surface impedance, it is convenient to present data by plotting the normalized impedance change $r = (Z_{cell}-Z_{no\ cell})/Z_{no\ cell}$. Figure 2c shows the predicted frequency dependence of r for a cell coverage of 90% of the electrode area.

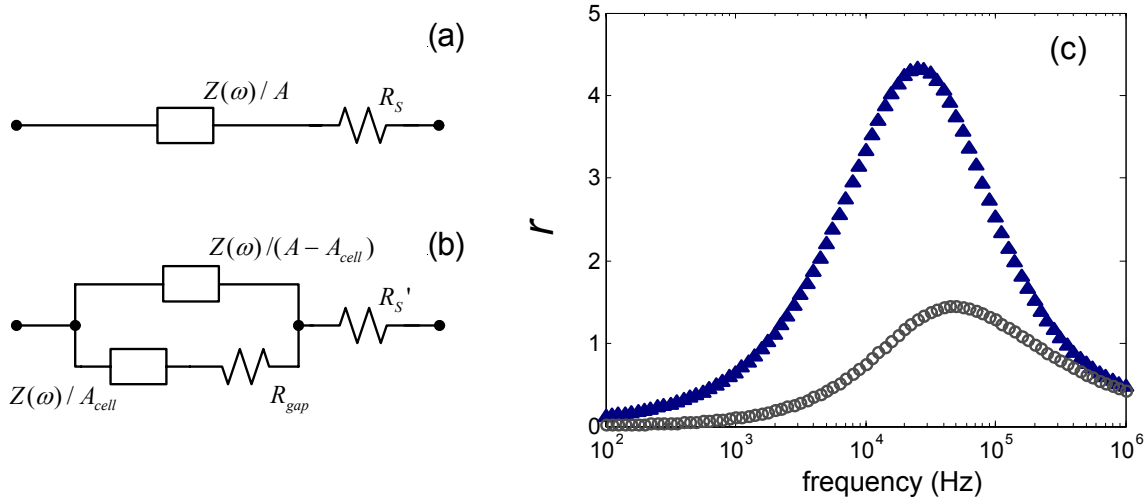

Figure 2. Effect of cells on electrode impedance: (a) equivalent circuit without cells; (b) equivalent circuit with cells; and (c) normalized impedance change for 90% coverage of 0.13 mm^2 electrode (○) 0.25 μm cell-electrode gap and (▲) 0.025 μm cell-electrode gap.

EXPERIMENTAL

Electrode arrays were fabricated using a lift-off process as previously reported [4]. We report here on measurements made using two different electrode array design [5].The electrodes were gold with a chromium adhesion layer and were fabricated on fused silica substrates. Parasitic capacitance between the interconnect lines and the liquid medium was reduced by painting the interconnect about 150 μm away from the electrodes with silicone EP30HT from Masterbond Inc. (Hackensack, NJ). Array A had a large grounded electrode in addition to a common electrode and four sensing electrodes ranging in area from 0.025 mm^2 to 0.44 mm^2 Array B had nine identical electrodes with exposed area between 0.015mm^2 and 0.02 mm^2 after painting. A bottomless plastic well cut from a 24-well culture dish was bonded onto the

substrate, enclosing the sensors and forming a chamber for cell culture. Array B had nine identical electrodes with exposed area between 0.015mm^2 and 0.02 mm^2.During the measurements, the well was placed in a temperature-controlled chamber mounted on an electronic probe station, with the chamber temperature controlled at 37 ± 1 °C. The impedance magnitude was measured as a function of frequency over the range from 100 Hz to 1 MHz using an HP 4192A impedance analyzer.

CELL ATTACHMENT AND GROWTH

Figure 3 shows the impedance as a function of time after deposition of 3T3 mouse fibroblasts. Over this time period, we observe the settling, adhesion, and spreading of the cells. As expected, the maximum normalized impedance change increases during this period. At the end of 5 hours the cell coverage observed by optical microscopy was about 95%.

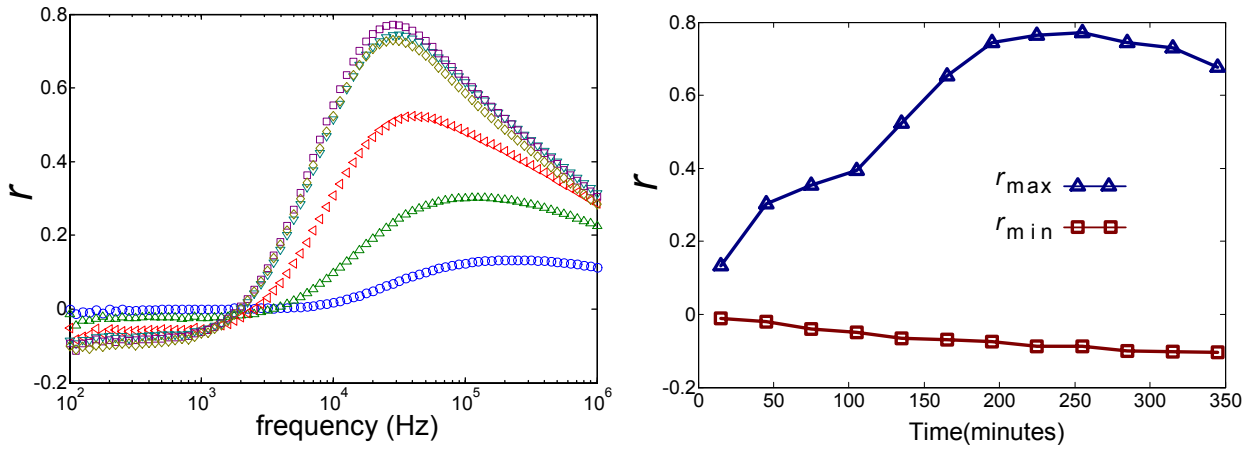

Figure 3. Plot of normalized impedance change (r) over 5 hours after 3T3 fibroblasts were deposited: (○) 15 min, (△) 0.5 hr, (◁) 2 hr, (▽) 3 hr, (□) 4 hr, and (◇) 5 hr after deposition. 90% to 95% cell coverage on substrate was observed by optical microscope. The electrode area was 0.13 mm^2. (b). Normalized impedance peak magnitude (r_{max}) and magnitude at 300 Hz (r_{min}) as a function of time after cell deposition.

After nearly complete coverage is achieved, r continues to change in a way that depends on the cell type. Figure 4 shows the changes that occur for mouse fibroblasts over a longer time period. Attachment and spreading of the cells takes place over a ~ 6 hour time period as before. We then observe a drop in r indicating a decrease in total area covered, due to cell motion and relief from the surface. During the next 18 hours, migration induced by gaps between cells and some proliferation occur. There is then a monotonic increase in r over a period of 48 hours as the cells grow to cover most of the remaining open area resulting in the formation of a compact monolayer.

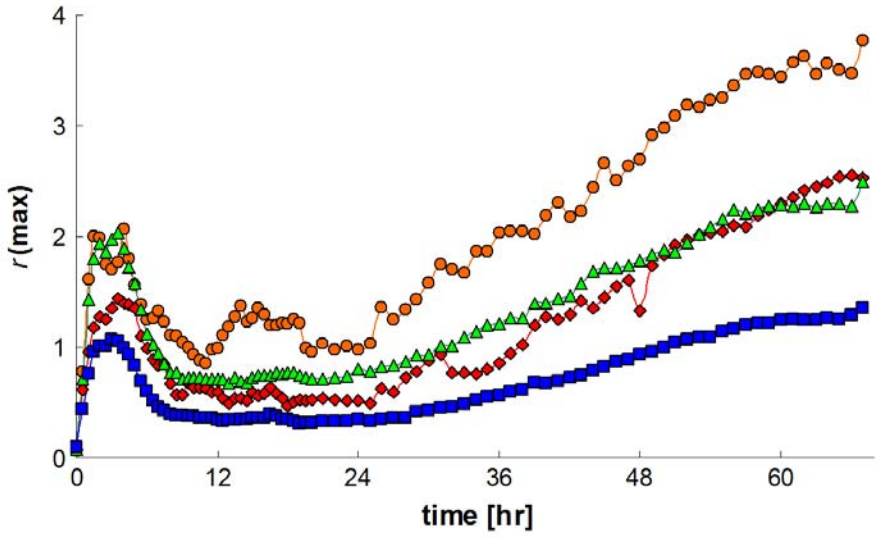

Figure 4. Plot of maximum *r* (normalized impedance change) as a function of time for fibroblast cells: (●0.017 mm^2; ◆ 0.037 mm^2; ▲0.09 mm^2 ■ 0.4 mm^2).

In contrast, different behavior is observed for human cancer cells. Figure 5 shows the normalized impedance change *r* as a function of time for HCT116 human cancer cells. Attachment and spreading occurs over a longer period of about 24 hours. Measurements over a longer time period show a near step-change in the impedance at about 30 hours after cells were introduced (not shown). We also observe a consistently lower peak frequency and a smaller value of r_{max} after the initial attachment period. This can be explained by the smaller size of cancer cells and/ or a smaller value for the cell-electrode separation.

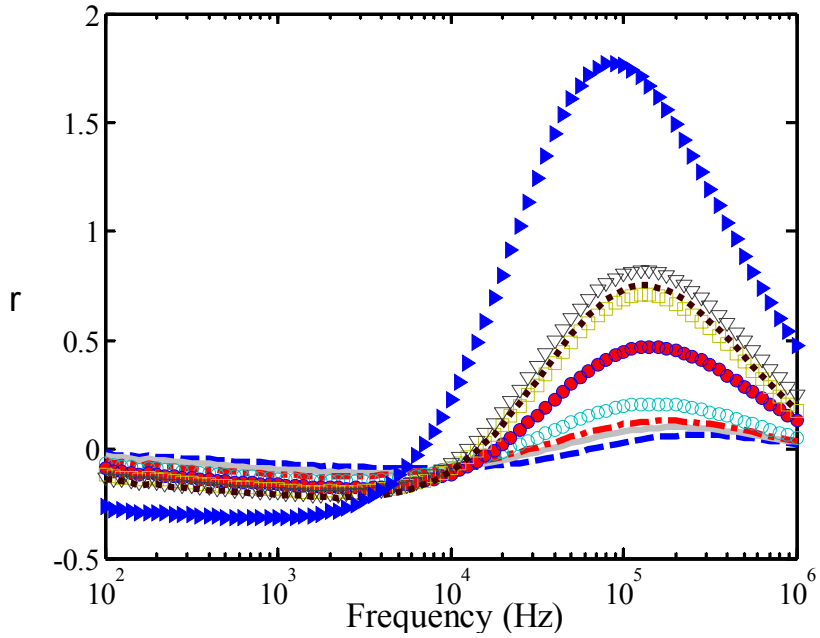

Figure 5. Plot of maximum *r* (normalized impedance change) as a function of time for HCT116 human cancer cells. (--) 45 min; (—) 2 hr; (— -) 4 hr; (○) 6 hr; (●) 10 hr; (□) 14 hr; (▪▪▪) 16 hr; (▽) 24 hr; and (▶) 48 hr.

The observed differences between normal and cancer cells indicate the potential for cell impedance measurements to provide useful diagnostic or prognostic information. Previous work has suggested that electrode impedance measurements can provide information about the micromotion of cells [2]. The micromotion is different for cancerous and normal cells [6,7] so it is possible that measurements of micromotion can be used diagnostically or prognostically. Impedance changes due to micromotion can be expected to partly average out when the electrode size is large enough to contain many cells. Consequently we seek to develop electrode arrays consisting of a large number of small electrodes. Small electrodes will exhibit larger changes in impedance due to micromotion and the availability of a large number of electrodes will make it possible to collect statistically significant information. In the following section, we discuss a possible design for an electrode array.

DESIGN OF ELECTRODE ARRAYS

Electrode arrays for cell impedance measurements have been previously proposed by Giaever and Keese [8]. This patent proposed arranging the electrodes in a matrix and then measuring the impedance between particular row and column lines to select the electrode at the crosspoint. This is a passive matrix array and suffers from "sneak paths" for large arrays. In order to fabricate a large array, we are using an active matrix design (Fig. 6a) in which a field effect transistor is used to select a particular electrode. For the electrode sizes of interest (30- 100 μm square to measure a single cell or a small cluster of cells), an array of hundreds of electrodes can be fabricated on a chip of a few mm^2 in size. Figure 6b shows a possible design for one site in the array. The array is designed in the MOSIS AMIS 1.5 micron process.

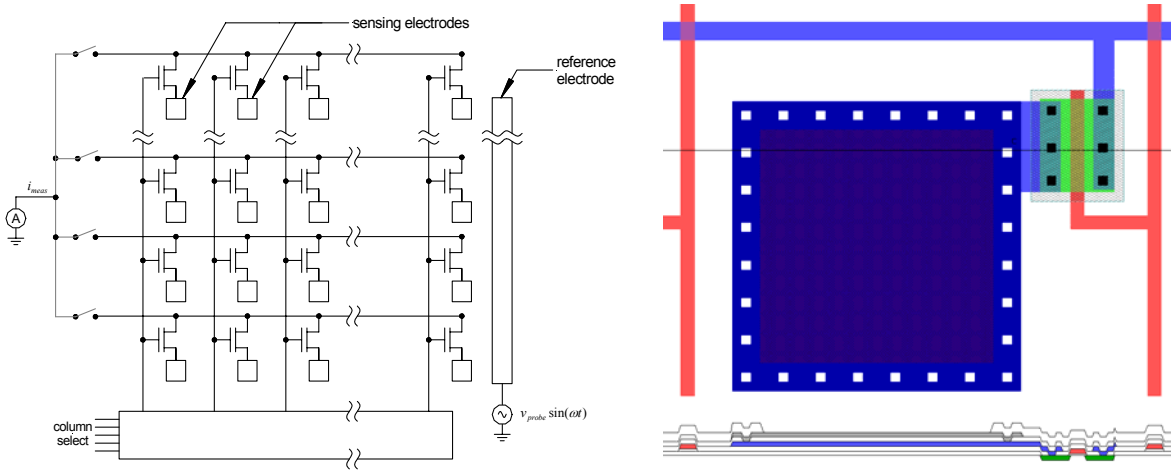

Figure 6. Active matrix sensor array: (left) circuit diagram of the array and (right) possible layout and cross section.

In this design, the electrodes are formed by using a glass cut to expose Metal2. As shown, the electrodes are 50 μm × 50 μm and can be designed with 50 μm spacing. Column lines are polysilicon and the row lines are Metal1. Note that postprocessing of the chip is required to coat the electrodes with gold and to passivate the bond pads and bond wires while exposing the sensing electrodes. Details of these aspects of the processing will be reported elsewhere. Here we focus on the design of the electrode array.

Referring to Fig. 3, we are interested in impedance measurements over the frequency range from approximately 10^3 Hz to 1 MHz. Over this range, the impedance of a 50 μm × 50 μm square electrode varies from approximately 410 kΩ to 6.4 kΩ ohms [4]. We require the ON resistance of the access transistors to be substantially less than this value. For a maximum error of 2%, this yields the condition

$$R = \frac{1}{2k_n' \frac{W}{L}\left(V_{GS} - V_T\right)} < 0.02 \cdot 6.4\ k\Omega$$

The AMIS 1.5 micron process has $k_n' \approx 36\ \mu A/V^2$ so at V_{GS}-V_T = 4.5 V this condition can be satisfied by a transistor with $W/L = 24$. A second condition arises because of capacitive coupling between the Metal1 row lines and the cell growth medium. This capacitance is approximately given by

$$C_{stray} = \frac{\varepsilon_i A}{t_i}$$

where A is the total area of the Metal1 row line and ε_i and t_i are the effective permittivity and thickness of the interlevel dielectric plus the passivation insulators, respectively. For a Metal1 line 3 μm in width and 3000 μm long (corresponding to 30 sensing sites) we estimate[1] $C_{stray} <$ 0.31 pF. We require that the impedance magnitude of the stray capacitance be substantially greater than that of the impedance of the sensing electrode. This condition is easily satisfied for an array with 30 sensing sites along a column line.

This analysis suggests that a chip with 900 sensing sites could be fabricated in this process with acceptable parasitics. Such a chip would have a sensing array 3 mm × 3 mm and might have a total area of approximately 16 mm^2 to allow for demultiplexing circuitry and bonding pads. Postprocessing and testing of a chip with about 120 sensing sites is presently under way.

SUMMARY

We have presented measurements of the impedance of cell-covered electrodes which show that there are easily observable changes which can be related to the attachment and proliferation of the cells. These observed impedance changes depend on the cell type. We have also presented a possible design for an active-matrix array of a large number of sensing sites.

ACKNOWLEDGEMENTS

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[1] Assuming an insulator thickness at least as great as 1 μm and the same dielectric constant as SiO_2.

REFERENCES

1. I. Giaever and C.R. Keese, *IEEE Transactions on Biomedical Engineering* **BME-33**, 242-247 (1986).
2. I. Giaever and C.R. Keese, *Proc. Natl. Acad. Sci.*, **88**, 7896-7900 (1991).
3. X. Huang, D. Nguyen, D.W. Greve, and M.M. Domach, (to be published in *IEEE Sensors*).
4. X. Huang, D.W. Greve, D. Nguyen, and M.M. Domach, in *Proc. IEEE Int. Conf. Sensors 2003*, pp. 305-309 (IEEE, Piscataway, NJ, 2004).
5. D.D. Nguyen, X. Huang, D.W. Greve, and M. M. Domach, (*Biotechnology and Bioengineering*, in press).
6. A.W. Partin, J.T. Isaacs, B. Treiger and D.S. Coffey, *Cancer Research* **48** 6050-3 (1988).
7. J.J. Latimer, in *Developing Technologies for Early Detection of Breast Cancer*, L. Newman, ed., summary of the workshop Committee on the Early Detection of Breast Cancer, pp. 14-15, National Cancer Policy Board, Institute of Medicine, (Washington, DC, 2000).
8. I. Giaever and C.R. Keese, "Cell substrate electrical impedance sensor with multiple electrode array," US patent 5,187,096 (1993).