Electrical Cell-substrate Impedance Sensing (ECIS) based Biosensor for Characterization of DF-1 Cells

H.R. Siddiquei, A. N. Nordin*, M.I. Ibrahimy
ECE Department,
Kulliyah of Engineering
International Islamic University
Kuala Lumpur, Malaysia

M.A. Arifin, N.H. Sulong , M. Mel
Biotechnology Engineering Department
Kulliyah of Engineering
International Islamic University
Kuala Lumpur, Malaysia

I. Voiculescu
Dept. of Mechanical Engineering,
City College of New York
160 Convent Avenue,
New York, USA

E-mail: anisnn@iium.edu.my

Abstract -Electric cell-substrate impedance sensing (ECIS) method can be used as a valuable tool for real time monitoring of cell behavior such as attachment, mobility, and growth. Changes in impedance of the cells due to growth and attachment can be modeled as an equivalent circuit consisting of resistors and capacitors of both the cell culture media and the cells. In this work, a biosensor which measures the impedance in DF-1 cells (derived from chicken embroyonic fibroblasts and CEF cells) are presented. The biosensor consists of a Teflon cell holder and two gold electrodes. Experimental measurements were conducted using DF-1 cells cultured in DMEM media. Two different experiments were conducted namely; the control experiment (holder contains only DMEM media) and the cell experiment (holder contains both DMEM media and cells). The biosensor was placed in an incubator with optimum settings for cell growth. Impedance measurements were sampled at six hour intervals. Based on these measurements the resistance and capacitance change due to the growth of the DF-1 cells were calculated. It was observed that significant change in resistance and capacitance values occurs in the first six hours, where cell growth and attachment is most active. After this period of time, the cells become confluent and capacitance values become saturated.

Keywords: Electrical cell impedance sensing (ECIS), DF-1, impedance spectroscopy, animal cell, electrode impedance.

I. INTRODUCTION

Impedance measurements of cells using miniature electrodes can provide crucial information regarding the cell's proliferation, morphology and motility [2]. Cell-based sensing techniques provide continuous and real-time measurements making it more efficient compared to existing methods of microscopic observation [3, 4]. Unlike conventional biosensors that use attached-affinity recognition molecules (e.g. antibodies), cell-based biosensors use living cells, which have a variety of native biomolecules on their surfaces [5]. These sensors rely mainly on fluorescence and electronic detection for sensing various cellular events, while cell-electronic sensors are often based on impedance measurement.

The operating principle of the Electrical Cell-substrate Impedance Sensing (ECIS) technique relates normalized measured impedance change to cell coverage, size of cell-substrate contacts and spaces. Typically, small gold electrodes are immersed in a tissue culture medium [1]. When cells get attached and spread on the electrodes, the impedance measured across the electrodes changes. This changing impedance can be used for understanding the cell behavior in the culture medium.

In our experiment, DF-1 cells (derived from chicken embryonic fibroblasts, CEF cells) were cultured in Dulbecco's Modification of Eagle's Medium (DMEM) media. The cells' growth rate was monitored over a period of 3 days by continuous impedance measurements using an impedance analyzer. This measuring method provides several advantages: (i) it is less time consuming compared to conventional methods, (ii) it is possible to automate and quantify cell morphology measurements, and (iii) the fluctuating impedance pattern can be used as signature for a cell [1]. This paper is organized as follows. Section II discusses the fundamental concepts of biosensors. Experimental setup and measurement procedure are briefly described in section III. Section IV contains experimental results and discussion. Finally conclusions are drawn in section V.

II. IMPEDANCE SENSING USING BIOSENSORS

A biosensor that utilizes impedance sensing typically consists of two metal electrodes: one large common reference electrode and one small working electrode. The cells were cultured in a holder and measured using gold electrodes. During culture, attachment and spreading behavior of cells on the electrode surface change the impedance in such a way that

This work was supported in part by the NSF-EAGER award number 0940111, Brain Gain Malaysia Incentive: MOSTI/BGM/R&D/500-2/6(2) and the Fundamental Research Grant: FRGS 0308-90 provided by the Ministry of Higher Education of Malaysia.

978-1-4244-6235-3/10/$26.00 ©2010 IEEE

morphological information of the attached cells can be inferred and so, these behaviors of the cells are important factors for this type of biosensor [6]. Suspension cells can usually grow and reproduce (*mitosis*) freely in a medium without being attached to any substrate/surface [1, 7]. Adherent cells, on the other hand, need to be attached to a surface before they grow and proliferate. After attachment the shape of the cells becomes flat and no longer remains spherical [1]. Fig. 1 illustrates this cell behavior in tissue culture medium.

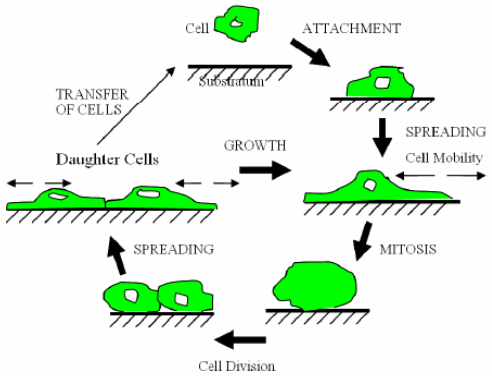

Figure 1. Cell behavior in tissue culture medium [1]

The initial impedance of the DMEM cell culture medium is measured first. The electrodes are immersed in an ionic DMEM cell culture medium with high conductivity ~ 0.015 Ω^{-1} cm^{-1}, which forms a resistor, R_{med} [5, 8, 9]. The DMEM cell culture medium also creates an electrified double layer or a capacitance representing the dielectric behavior of the medium or C_{med} [4, 8]. The series network of R_{med} and C_{med} forms the equivalent circuit without cells is as shown in Fig.2.

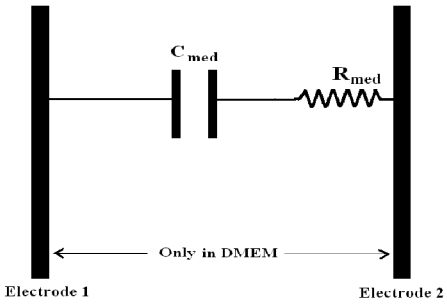

Figure 2. Equivalent circuit of electrode without cells

When cells are inoculated into the culture medium, the presence of cells in the holder and its growth causes changes of electrode impedance [4]. The impedance change due to cell growth is described as

$$Z_{cell} = \frac{Z - Z_{initial}}{Z_{initial}} \qquad (1)$$

Where $Z_{initial} = R_{med} + jX_{C_{med}}$ and $Z = R + jX$ [10, 11]. DF-1 cells are typically poorly conductive membranes which exhibit capacitance of ~ 1μF/cm^2 [9]. The total impedance is a combination of the cells' capacitance denoted as C_{cell} and the resistance of the chicken cell, R_{cell}. Change in impedance values are observed when there is addition, movement or growth of cells. The equivalent circuit model for the impedance of the DF-1 cells inoculated in DMEM media is shown in Fig. 3.This total impedance, Z can be also be described using

$$Z = R_{med} + \frac{\left(X_{C_{cell}} + R_{cell}\right) X_{C_{med}}}{X_{C_{cell}} + X_{C_{med}} + R_{cell}} \qquad (2)$$

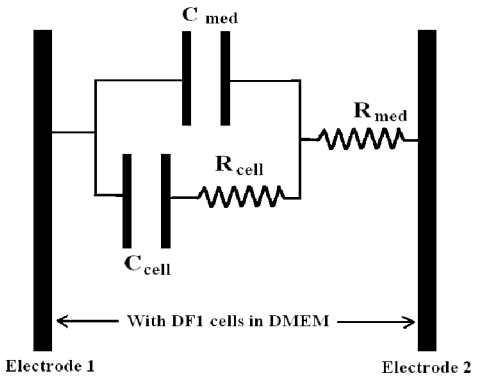

Figure 3. Equivalent circuit of DF-1 cells in DMEM

III. Experimental Work

The measurement system consists of an Agilent Impedance analyzer 4294A connected to two gold electrodes using coaxial cables (Fig. 4 and 5). The electrodes are placed inside a Teflon holder. All procedures for the preparation of DMEM media and cells were carried out aseptically in a biohazard safety hood. The impedance of the DMEM media inside the holder was measured first. Next, 1.0×10^5cells/ml of DF-1 cells were inoculated in the culture medium and injected into the holder. To provide suitable environment for cell growth, the biosensor (holder and electrodes) was placed inside an incubator. The temperature of the incubator was kept at 37°C and 5% CO_2 gas (v/v) was provided. Impedance measurements readings were taken every 6 hours for a total period of 54 hours. Fig. 6 is a microscopic image of the DF-1 cells in confluent state.

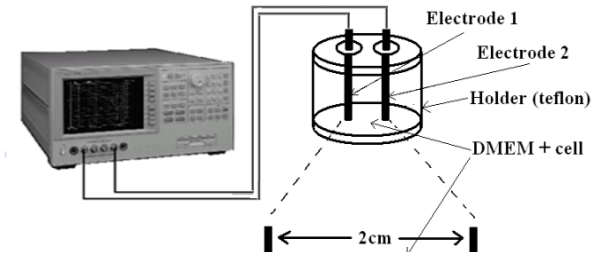

Figure 4 Schematic of experimental Setup

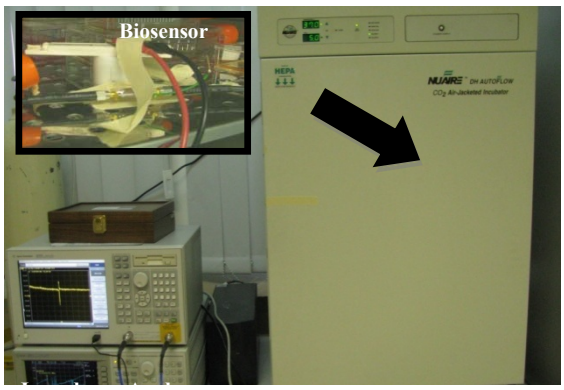

Figure 5. Snapshot of actual experimental setup

IV. Results and Discussion

The impedance analyzer provides measurements of both the impedance (Z) and the phase angle (Θ). Impedance measurements between the two gold electrodes were taken at six hour intervals over a wide frequency range from 0.5 Hz to 15 MHz. Fig. 7 illustrates the change in impedance measurements (*Z*) over this frequency range. Two measurements are illustrated in this figure, namely impedance of the cells after 6 hours and 54 hours of incubation respectively. It can be concluded that the change in impedance were due to the growth of DF-1 cells. The noise in the impedance is produced by the magnetic stirrer inside to incubator.

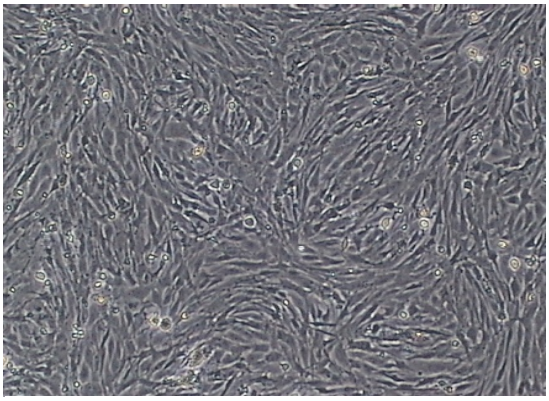

Figure 6. DF-1 cells during confluent state

It can be seen in Fig. 7 that a marked change in impedance occurs at the higher frequency range (MHz). In this work, we have chosen to calculate the impedance of the cells at 9.927 MHz. The measured overall impedance at a specific frequency of 9.927 MHz and time *t* is denoted as

$$Z = R + jX \qquad (3)$$

Based on the real component of (3), the expression for the resistance of the cells, R_{cell} can be described as

$$R_{cell} = R - R_{med} \qquad (4)$$

The overall capacitance due to the presence of cells and DMEM measured between the two electrodes can be denoted as

$$C = \frac{1}{2\pi f X} \qquad (5)$$

Based on the model shown in Fig. 3, the impedance of the cell can be described as

$$X_{C_{cell}} = \frac{(Z-R_{med})\left(X_{C_{med}}+R_{cell}\right)-X_{C_{med}}.R_{cell}}{X_{C_{med}}-Z+R_{med}} \qquad (6)$$

Using equations (3) to (6) the overall resistance, impedance and capacitance can be calculated and summarized in Table 1. Table 1 also shows the calculated resistance of the cells, R_{cell} and impedance of the cells, X_{Ccell}.

From this table, it can be seen that the overall resistance between electrodes increased from $R_S(0)$ = 270.804Ω to $R_S(54)$ = 413.821 Ω. The difference in resistance due to the

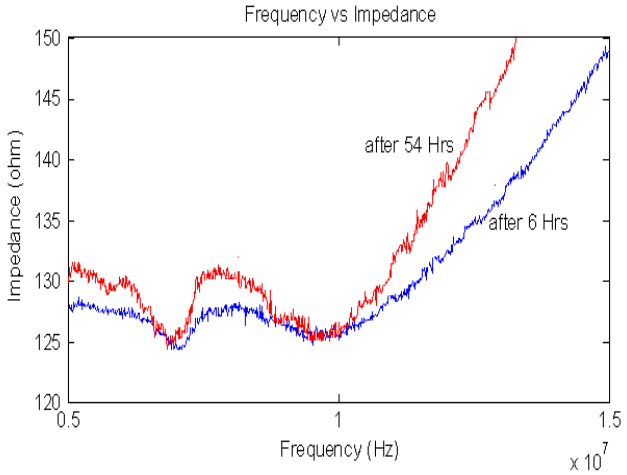

Figure 7. Impedance measurements with growth of cells

growth of cells can be calculated as $R_S(54)$ - $R_S(0)$ = 143.07 Ω. Hourly change of resistance only due to cell growth and death is shown in 5th column of the above table. Based on these measurements, it can be concluded that the highest cell growth occurs during the first six hours with the highest increase of resistance, $R_{cell}(6)$ = 238.956Ω.

This observation is also supported by a plot of overall capacitance versus time shown in Fig. 8. From this figure, it can be seen that when the DF-1 cells attach rapidly to the surface of the electrodes, impedance increases and capacitance decreases. This phenomenon is due to the increase in the number of cells, which correspondingly increases the total resistance since each DF-1 cell contributes a small resistance. The attachment and arrangement of cells at the surface can be visualized as a series connection of these small resistances. In contrast, a series connection of the capacitance due to each cell would decrease the overall capacitance. After a certain time the growth and attachment rate to the electrodes surface reduces and saturates.

TABLE I. OVERALL AND CELL RESISTANCES AND IMPEDANCES

Time (Hrs)	R (Ω)	X (Ω)	C (nF)	R_{cell} (Ω)	X_{Ccell}(Ω)
0	270.804	62.677	0.2557	0	0
6	509.760	322.585	0.0497	238.956	259.908
12	401.701	212.430	0.0754	- 108.06	-110.15
18	416.051	236.674	0.0677	14.350	24.243
24	425.463	251.309	0.0638	9.412	14.635
30	423.354	233.860	0.0685	-2.109	-17.448
36	423.928	247.093	0.0649	0.574	13.233
42	403.835	210.596	0.0761	-20.093	-36.497
48	420.593	248.735	0.0644	16.758	38.139
54	413.821	210.535	0.0761	-6.772	-38.200

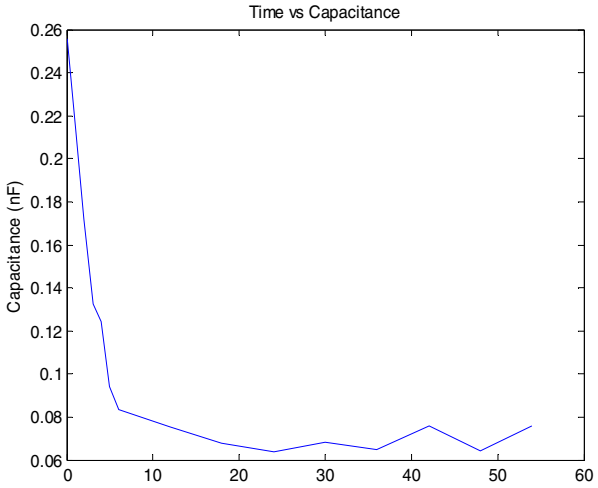

Figure 8. Time course of overall Capacitance at 9.927 MHz

V. CONCLUSIONS

In this work, it has been demonstrated that change in the DF1 cell physiology causes large, easily observable changes in the electrical impedance. The peak magnitude and the position of the normalized impedance change were found to be a function of cell parameters: cell attachment, cell growth and cell coverage.

REFERENCES

[1] S. P. Mohanty, "Biosensors: A Survey Report," Citeseer, 2001.

[2] I. Giaever and C. R. Keese, "Use of Electric Fields to Monitor the Dynamical Aspect of Cell Behavior in Tissue Culture," *Biomedical Engineering, IEEE Transactions on*, vol. BME-33, pp. 242-247, 1986.

[3] D. J. Stephens and V. J. Allan, "Light microscopy techniques for live cell imaging," *Science*, vol. 300, pp. 82, 2003.

[4] X. Huang, D. W. Greve, D. D. Nguyen, and M. M. Domach, "Impedance based biosensor array for monitoring mammalian cell behavior," presented at Sensors, 2003. Proceedings of IEEE, 2003.

[5] L. Huang, L. Xie, J. M. Boyd, and X. F. Li, "Cell-electronic sensing of particle-induced cellular responses," *The Analyst*, vol. 133, pp. 643-648, 2008.

[6] J. Wegener, C. R. Keese, and I. Giaever, "Electric cell–substrate impedance sensing (ECIS) as a noninvasive means to monitor the kinetics of cell spreading to artificial surfaces," Experimental cell research, vol. 259, pp. 158-166, 2000.

[7] A. Han, L. Yang, and A. B. Frazier, "Quantification of the heterogeneity in breast cancer cell lines using whole-cell impedance spectroscopy," Clinical Cancer Research, vol. 13, pp. 139, 2007.

[8] S. M. Radke and E. C. Alocilja, "Design and fabrication of a microimpedance biosensor for bacterial detection," Sensors Journal, IEEE, vol. 4, pp. 434-440, 2004.

[9] X. Huang, D. Nguyen, D. W. Greve, and M. M. Domach, "Simulation of microelectrode impedance changes due to cell growth," IEEE Sensors Journal, vol. 4, pp. 576-583, 2004.

[10] Y. Qiu, R. Liao, and X. Zhang, "Impedance-Based Monitoring of Ongoing Cardiomyocyte Death Induced by Tumor Necrosis Factor-[alpha]," Biophysical Journal, vol. 96, pp. 1985-1991, 2009.

[11] D. B. Hernandez, "Development of a Software Tool for Electrical Bioimpedance Spectroscopy Analysis," in Biomedical Engineering: Hogskolan Iboras, 2008.