JOURNAL OF PURE AND APPLIED MICROBIOLOGY, May 2014. Vol. **8**(Spl. Edn. 1), p. 827-831

The Study of Cell Attachment and Spreading on Polyaniline and Gelatin using Electric Cell-substrate Impedance Sensing

Irmanisha Ibrahim[1], Anis Nurashikin Nordin[2]* and Yumi Zuhanis Has-Yun Hashim[1]

[1]Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia, P.O Box 10, 50728, Kuala Lumpur, Malaysia.
[2]Department of Electrical and Computer Engineering, Faculty of Engineering, International Islamic University Malaysia, P.O Box 10, 50728, Kuala Lumpur, Malaysia.

(Received: 08 January 2014; accepted: 24 March 2014)

Electrical cell-substrate impedance sensing (ECIS) is a valuable tool for real time monitoring of cell behavior such as attachment, mobility, and growth. This study investigates the correlation between cell attachment and impedance when cells are attached to two different substrates, polyaniline and gelatin, respectively. Colon cancer cell lines, HCT-116 were used as model cell line. The impedance measurements were measured every 8 hours for 104 hours at frequency of 40Hz to 10MHz using impedance analyzer. Polyaniline produced a graph that is in agreement with typical growth curve for mammalian cell culture at lag, and early log phase. However, gelatin graph showed a different trend. This may be due to the high conductivity of the polyaniline which gave better cell attachment and spreading for the HCT-116 cells. The efficiency of the biosensor was measured by cytotoxicity test using 2.5 μg/ml 5-FU and the changes on impedance were analyzed. In conclusion, the cell attachment correlates with impedance depending on the substrate used to culture the cells. To this end, ECIS is proven as an alternative tool to measure cell behavior with an added advantage of ability to monitor the progress in real-time and showed a great potential in drug testing application.

Key words: ECIS; Impedance Biosensor; Drug testing; HCT-116; Cell Attachment.

In vitro cytotoxicity assays are alternative methods to animal testing to determine basal cytotoxicity or assess cell viability assessment, by measuring of the number of live cells or dead, after exposure to chemicals[1, 2]. Tetrazolium dye (MTT) colorimetric assay, sulforhodamine B (SRB) colorimetric assay and Adenosine Triphosphate (ATP) measurements are few of the conventional cytotoxicity assays that have been used to investigate the effect of drug or toxin on cells[2].

However, there are several limitations of these conventional assays. For instance, they are based on single-end point detection which cannot be used many times. Besides, they are also time consuming, complicated, invasive, and expensive[3, 4]. Therefore, there is a strong demand for label-free detection methods for cell-based assays[3]. In this case, cell-based impedance biosensor has been an emerging tool in studying animal cell culture behavior that can be used to non-invasively and instantaneously detect and analyze cell responses to chemical and biological agents[4].This is due to its advantages in getting measurement in real time monitoring apart from being label-free, easy, low cost, sensitive and invasive. Besides that, impedance biosensor can also act as a tool to satisfy some process analytical technology (PAT)

* To whom all correspondence should be addressed.
Tel.:+ 603-6196 447;
E-mail: anisnn@iium.edu.my

requirement[5]. One of the established cell-based impedance biosensor is Electrical Cell-Substrate Impedance Sensing (ECIS).

ECIS is a term coined by Giaever and Keese that refers to the study of cells based on their attachment to the substrate (electrodes)[6]. In cancer cells study, ECIS has been used to monitor the changes in the behavior of the cells such as their attachment, mobility and spreading in terms of their changes in the impedance measurement. When cells get attached and spread on the electrodes, the impedance measured across the electrodes changes. This changing impedance can be used to understand cell behavior in the culture medium[7].

The aim of this study was to design and fabricate the electrode, and consequently to study the cell attachment on two different substrates (i) polyaniline; a conductive polymer, and (ii) gelatin; a natural biological protein. Lastly, by using the optimum frequency achieved, the biosensor was used to investigate the feasibility of the electric cell-substrate impedance sensing for drug testing applications.

MATERIALS AND METHODS

Polyaniline (emeraldine base) was purchased from Aldrich Chemistry (Sigma Aldrich, USA), bovine gelatin was purchased from Gibco (Paisley, UK) and N-Methyl-2-pyrrolidinone (NMP) was purchased from (Sigma Aldrich, USA). Meanwhile, the anticancer drug, Fluorouracil or 5-FU was purchased from Fluka Analytical (Sigma Aldrich, USA). All other chemicals used in this study were obtained commercially as reagent grade.

Cell lines and culture

Human colon cancer cells (HCT-116, ATCC®CCL-247) were obtained from Sigma-Aldrich (Munich, Germany). The HCT-116 cells were maintained in the tissue culture flasks in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% antibiotic penicillin/streptomycin in a humidified incubator at 37ÚC in an atmosphere of 95% air and 5% CO_2. The medium was changed and cells were passaged using accutase once the cells reached 80% confluency[8].

Electrode fabrication

The electrode was designed by using Paint Software. It consisted of two types of electrodes(i) working electrode in which the reaction of interest occurs, and (ii) counter electrode thatallows the electric current to flow to the working electrodes. The electrode designed (Figure 1) consisted of eight copper electrodes that were printed on positive printed circuit board by using semiconductor technology. The completed board was then tested using discontinuity process to check for any short circuit.Prior to cell attachment, this electrode was attached with 8 well cultureware and coated with two different attachment factors; polyaniline and gelatin.

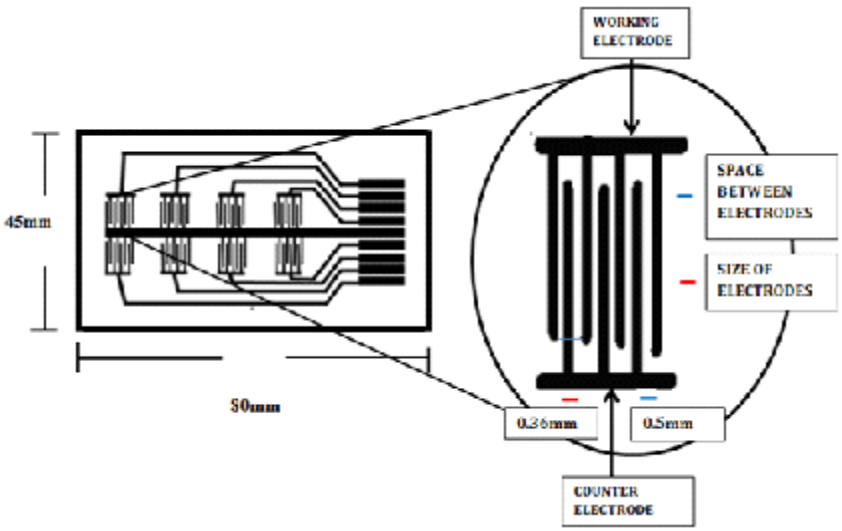

Fig. 1. Circuit electrode design using Paint Software

Preparation of the coating film

0.1% (w/v) polyaniline (PANi) and 0.1% (w/v) gelatin concentration were tested in this experiment. The PANi solution was prepared by mixing the PANi-emeraldine base with N-methyl-2-pyrrolidinone (NMP)[9]. Meanwhile, the gelatin solution was prepared by mixing the gelatin powder with deionized water. The polyaniline and gelatin films were prepared by coating them directly on the electrode surface of the impedance spectroscopy biosensor. The access solution was aspirated and washed using phosphate buffer saline (PBS) prior to cell loading.

Impedance measurement

The impedance was measured at frequency of 40Hz to 10MHz for 104 hours with 8 hour intervals using the Agilent Precision Impedance Analyzer 4294A. The results were then analyzed by plotting a graph as a function of impedance versus time at different frequency ranges.

Sulforhodamine B (SRB) assay

The half maximal (IC_{50}) effect of 5-FU on HCT-116 cell lines was determined by the sulforhodamine B (SRB) protein stain assays as previously described [10, 11, 12]. Briefly, the cells were plated (190 µl per well) in 96-well micro-plates at densities of 100,000 cells per well. About 10 µl of 5-FU with different concentrations were added into the well after 72 hour incubations. Negative control wells containing the same volume of cells with 10 µl DMSO and Blank sample with 200 µl per well of complete culture medium were also included in this experiment. Fixation procedure was carried out after the micro-plates were left for another 72 hours at 37ÚC to enable the cells to attach to the bottom of the wells. About 100µl of 50% *Trichloroacetic acid(*TCA) was added into each well and the plates were incubated at 4°C for 1 hour prior to washing with tap water and left at room temperature for a day to make sure the plates were completely dried. Then it was stained with 100 µl of SRB dye (0.057 % wt/vol). The plates were incubated at room temperature for another 30 minutes, washed with 1% acetic acid and left at room temperature for a day or until dried. Absorbance measurement was performed at 510 nm after the wells were solubilized for 30 minutes at room temperature with 200 µl of 10 mM, pH 5 Tris base solution. The IC_{50} value for 5 FU on HCT-116 cells was plotted in the function of 5 FU concentrations.

Feasibility study as alternative method for cytotoxicity assay

The SRB assay was conducted as described in section 2.6 in order to get the half maximal inhibitory concentrations (IC_{50}) of 5-FU on HCT-116. The obtained IC_{50} concentration was tested on the HCT-116 cell linesusing the impedance analyzer with polyaniline coating at 40 Hz. The impedance readings were taken at 8 hour intervals for 104 hours.

RESULTS AND DISCUSSION

The fabricated electrode is shown in Figure 2. Higher impedance measurement was obtained from polyaniline substrate, compared to gelatin. This suggests that polyaniline was a better substrate for the biosensor. Meanwhile, the optimum frequency obtained from the untreated cells impedance measurement gave 40Hz as the most sensitive frequency to detect the behavior changes of the cancer cells.

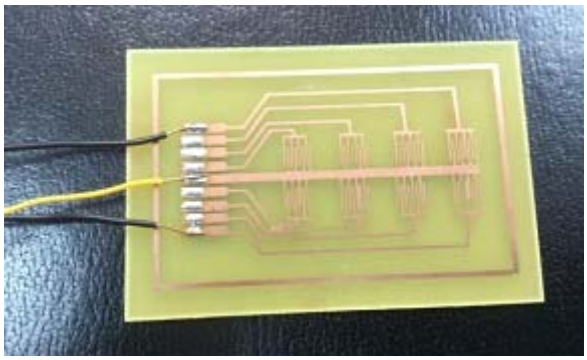

Fig. 2. The fabricated electrode

From Figure 3 and 4, cell attachment measured in the function of impedance (Z) was gelatin less than polyaniline(1500 Ω compared to 4500 Ω respectively). Therefore, polyaniline was chosen as the material for the ECIS biosensor for colon cancer cells with 40 Hz as the optimum frequency.

In order to test for the efficiency of the impedance biosensor, cytotoxicity test was done. From the SRB assay, the IC_{50} of 5-FU on HCT-116 cells was approximately 2.5 µg/ml. The impedance measurement results (Figure 5) of the treated samples (HCT-116 with 5-FU) showed a decrease in the impedance values (140 – 180 Ω) as compared to untreated cells (1000 – 4500 Ω). This showed that the fabricated biosensor is efficient and compatible to be used in drug testing applications.

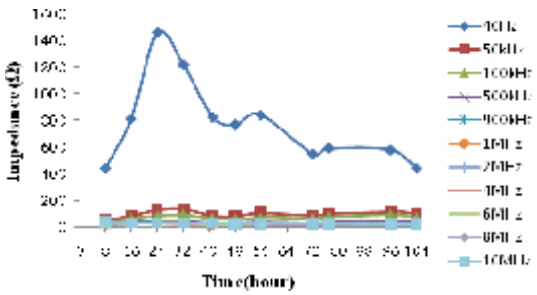

Fig. 3. Graphs Impedance versus Time for Gelatin Coating

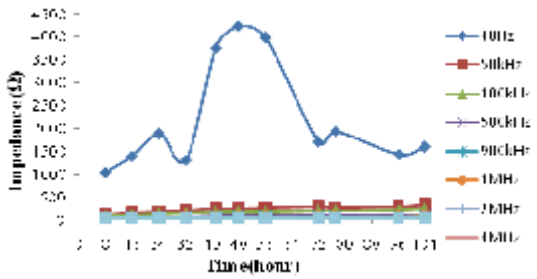

Fig. 4. Graphs Impedance versus Time for Polyaniline Coating

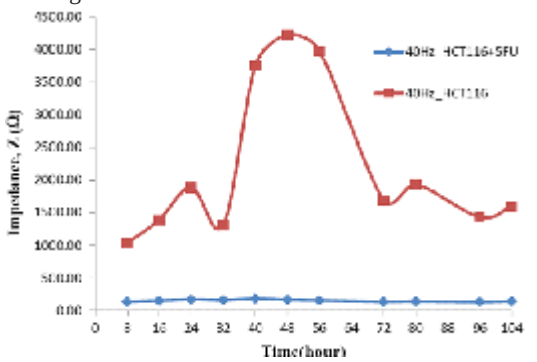

Fig. 5. Graphimpedance results comparisons between the untreated samples (HCT-116 cells) and treated samples (HCT-116 cells with 2.5ug/m l5-FU) with Polyaniline Coating at 40Hz

CONCLUSIONS

In conclusion, the cell attachment correlates with impedance depending on the substrate used to culture the cells. The biosensor also has the potential to be used as an alternative tool for drug testing procedure.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the funding support from the Ministry of Science and Technology (MOSTI) e-Science Research Grant (SF12-019-0048) and Prototype Research Grant Scheme (PRGS 12-010-0010).

REFERENCES

1. Zucco, F., Angelis, I. D., Testai, E., &Stammati, A. Toxicology Investigation with Cell Culture Systems: 20 Years After. *Toxicol. In Vitro*, 2004; **18**: 153-163.
2. Ceriotti, L., Ponti, J., Broggi, F., A.Kob, Drechsler, S., Thedinga, E., *et al*. Real-Time Assessment of Cytotoxicity by Impedance Measurement on a 96-well Plate. *Sensors and Actuators B.*, 2007; **123**: pp769–778.
3. Hug, T. Biophysical Methods for Monitoring Cell-Substrate Interactions. Assay Drug *Dev. Technol*, 2003; **1**: 479-488.
4. Asphahani, F., & Zhang, M. Cellular Impedance Biosensors for Drug Screening and Toxin Detection. *Analyst. Author Manuscript*, 2004.
5. Justice, C., Brix, A., Freimark, D., Kraume, M., Pfromm, P., Eichenmueller, B., et al. Process Control in Cell Culture Technology Using Dielectric Spectroscopy. *Biotechnol. Adv.*, 2011; **29**: 391-401.
6. Wegener, J., Keese, C., & Giaever, I. Electric cell-substrate impedance sensing (ECIS) as a noninvasive means to monitor the kinetics of cell spreading to artificial surfaces. *Exp. Cell Re.*, 2000; 158-166.
7. Siddiquei, H. R., Nordin, A. N., Ibrahimy, M. I., Ariffin, M. A., Sulong, N. H., Mel, M., et al. Electrical Cell-substrate Impedance Sensing (ECIS) based Biosensor for Characterization of

DF-1 cells. *International Conference on Computer and Communication Engineering (ICCCE 2010)*. Kuala Lumpur, Malaysia: Institute of Electrical and Electronics Engineers (IEEE), 2010.

8. Shakibaei, M., Mobasheri, A., lueders, C., Busch, f., Shayan, P., &Goel, A. Curcumin Enhances the Effect of Chemotheraphy Against Colorectal Cancer Cells by Inhibition of NF-KB and Src Protein Kinase Signaling Pathways. PLOS ONE, 2013; 8(2): e57218. doi:10.1371/journal.pone.0057218.
9. Bidez, P. R., Li, S., Macdiarmid, A. G., Venancio, E. C., Wei, y., & Leikes, P. I. Polyaniline, An Electroactive Polymer, Supperts Adhesion and Proliferation Pf Cardiac Myoblast. *J. Biomater. Sci. polymer. Edn*, 2006; pp 199-212.
10. Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S., Boyd, M.R.New Colorimetric Cytotoxicity Assay for Anti-Cancer Drug Screening. *J. Natl. Cancer. Inst.*, 1990; **82**: 1107.
11. Skehan, P. Assays of cell growth and cytotoxicity. In:.Studzinski Ed. , Cell Growth and Apoptosis. A Practical Approach. Oxford University Press, New York, 1995; p. 169.
12. Papazisis, K., Geromichalos, G., Dimitriadis, K., & Kortsaris, A. Optimization of the sulforhodamine B colorimetric assay. *J. Immunol. Methods.*, 1997; **208**: 151-158.