CONTINUOUS NON-DESTRUCTIVE MONITORING OF CELL HEALTH USING IMPEDANCE BASED INTERDIGITATED ELECTRODE STRUCTURED SENSORS

Anna Paschero, Eve McLoughlin and Eric Moore
(Tyndall National Institute, University College Cork, Cork, Ireland)

ABSTRACT: This article examines some preliminary tests which were performed in order to evaluate the best electrode configuration (width and spacing) for cell culture analyses. Biochips packaged with indium tin oxide (ITO) interdigitated electrodes (IDEs) were used to perform impedance measurements on A549 cells cultured on the surface of the biochip. Several tests were carried out using a 10mM solution of Sodium Chloride (NaCl), cell medium and the cell culture itself to characterize some of the configurations already fabricated in the facilities at Tyndall National Institute. The results show that the sensitivity changes using different width and spacing of the IDEs.

INTRODUCTION

Cell-based biosensors (CBBs), which use living cells as sensing elements, are able to provide functional information and quantitative analysis of biological cell. In general, they maintain living cells and observe the cellular physiological response after exposing the cells to different drugs (Asphahani and Zhang, 2007).

This particular project involves the use of bioimpedance measurements applied to cell cultures, as concentration, growth and alterations of the physiological state of cells during cultivation can be detected as impedance change.

The cell based biochips consist of finger electrodes composed of indium tin oxide (ITO). Indium tin oxide (or tin-doped indium oxide) is a mixture of indium(III) oxide (In_2O_3) and tin(IV) oxide (SnO_2) and when it is deposited as a thin film on glass or clear plastic it behaves as a transparent electrical conductor. ITO is used as an impedance sensor that allows real time non-invasive *in-vitro* analysis of the physiological state of biological cells due to its conductive, biocompatible and transparent characteristics. Investigation verified that growth of cells on indium tin oxide sensor surface was comparable with growth of cells in tissue culture flasks (Moore et al., 2009). ITO electrodes can therefore be considered a valid alternative to gold electrodes as they can provide both optical and electrical information during the experiment (Choi et al., 2007).

The experiments were conducted using different cell lines (e.g. A549, BALB3T3) cultured on the ITO electrode surface. When cells were placed on the electrode they blocked the current flow in a passive way and the bioimpedance increased. The current focus is the optimization of the electrode configuration, such as width and spacing, tailoring the sensor design to different cell types in order to determine if the surface architecture influences cell growth and hence cell response. Depending on the configuration, the electrode could show a different sensitivity (Alexander Jr et al., 2010).

Parallel optical analysis for real-time video monitoring was used to complement the impedance measurement, providing useful information on the cell behaviour, such as morphology and distribution

MATERIALS AND METHOD

Fabrication of ITO Electrodes. A Pyrex wafer was covered with a mask patterned with the design of the IDE and ITO powder was evaporated on it maintaining a thickness of 220nm.

For the packaging process the flip chip technology was used in order to interconnect the chips to a printed circuit board (PCB). Solder bumps were deposited onto the chip pads, then the chip was flipped (face down) and its pads were aligned to the PCB pads. The interconnection was completed by the solder flowing. Figure 1 shows a packaged biochip and the chip.

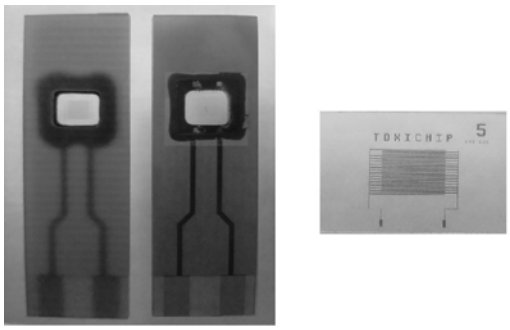

FIGURE 1. Packaged biochip and IDE on the Pyrex wafer

Nine different configurations were developed, changing the finger width and the finger gap (Table 1 and Figure 2).

Biochip Number	Finger Width (μm)	Finger Gap (μm)
1	60	10
2	60	20
3	60	40
4	40	10
5	40	20
6	40	40
7	20	10
8	20	20
9	20	60

TABLE 1. Electrodes configuration

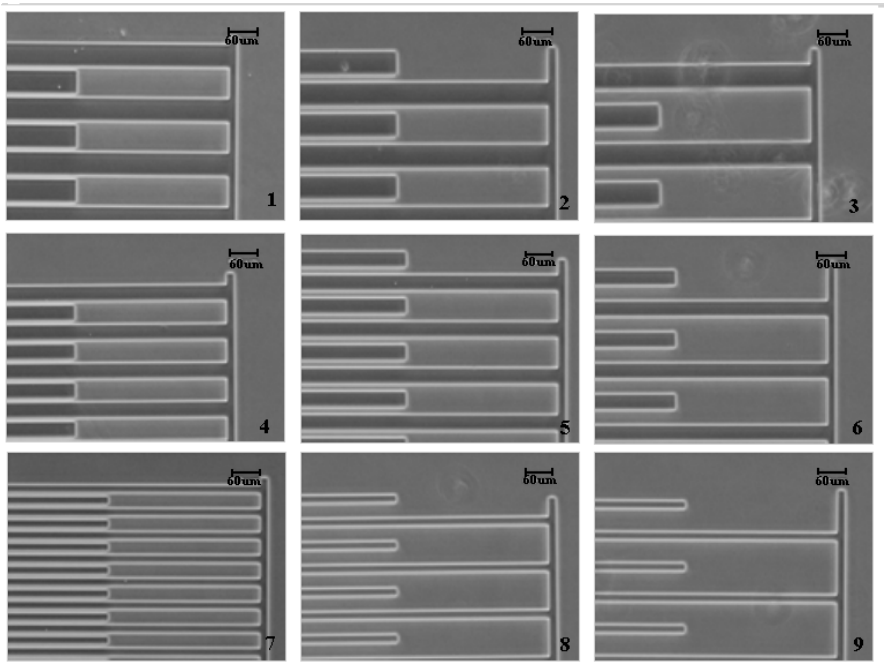

FIGURE 2. IDEs configurations

Cleaning Protocol. Before culturing the cells on the electrode surface, it was necessary to follow a cleaning protocol with the following steps: soak the biochips in isopropanol (IPA) for 20 minutes to dissolve any contamination due to the fabrication and packaging process and sonicate if necessary; use swaps soaked in IPA to manually remove any contamination if required. Wash the biochips in distilled water for 10 minutes (sonicate if necessary) and dry them in the fume hood using a nitrogen gun. After this process the biochips were oxygen plasma treated for 15 minutes at 100W in order to remove any further organic matter on the sensor surface; during this process the sensor needed to be exposed to the oxygen plasma (face up). Prior to tissue culture use the biochips were autoclaved at 121°C for 15 minutes and then stored under sterile conditions.

A549 Cell Culture Protocol. A549 lung tumour cells (ATCC) were routinely cultured in Dulbecco's Modified Eagle Medium (DMEM) under standard conditions (37^{o}C, 5% CO_2 in air, ≥95% humidity. The medium was supplemented with 1% gentamycin and 10% Foetal Bovine Serum (FBS) at final concentration. The cells were subcultured at 95% confluence and the medium was replenished every 2-3 days. Devices were cleaned following the cleaning procedure previously described. Cylinders were attached using silicon grease and 50µl of medium was used to coat the electrodes for 1 hour prior to cell seeding. The devices were then inoculated with approximately 70µl of cell suspension with a concentration of 1.5 x 10^5 cells/ml to give a final volume of 120µl of medium. An incubation period of 24 hours was maintained to ensure proper adherence of the cells to the electrodes prior to impedance measurements.

Impedance Measurement. Impedance measurements were performed using the Zahner IM6 Impedance Analyzer, an electrochemical workstation which combines high precision hardware and powerful, user-friendly software to simplify the acquisition and analysis of high quality, reliable impedance data.

The frequency range chosen for these tests was from 100Hz to 1MHz. Electrochemical impedance is usually measured by applying an AC potential to an electrochemical cell and measuring the current through the cell. For the experiments presented in this article the applied AC potential is 100mV.

A first measurements was performed using only medium, while the second one using A549 cells.

Optical Analysis. The images of the cells were taken using two different microscopes: a reverse microscope module and an inverted phase contrast microscope.

The first system used an USB camera chip module (CMOS technology) to acquire the image coming from the biochip under analysis. Focusing of the biosensor image was performed with the aid of a linear stage device. The data coming from the reverse microscope were collected in an USB HUB and then were sent to the PC station, where a specific control application processed this data and displayed the results. The biochip needed to be illuminated from the top, because the cell cultures were analyzed by transparency. The objective was a "MICRO 20× JIS" from Edmund Optics (20× magnification).

The second system was the compact inverted microscope for routine use Olympus CKX41 with a 100× magnification.

RESULTS AND DISCUSSION

Preliminary impedance tests were performed using 10mM NaCl solution in order to verify the repeatability of the results. The chips showing an error higher than 2% were not considered for the experiment.

Following the preliminary tests, measurements were performed with medium only, and with A549 cells cultured on the electrode surface. Figure 3 and 4 show the results obtained using biochip #2 and biochip #6.

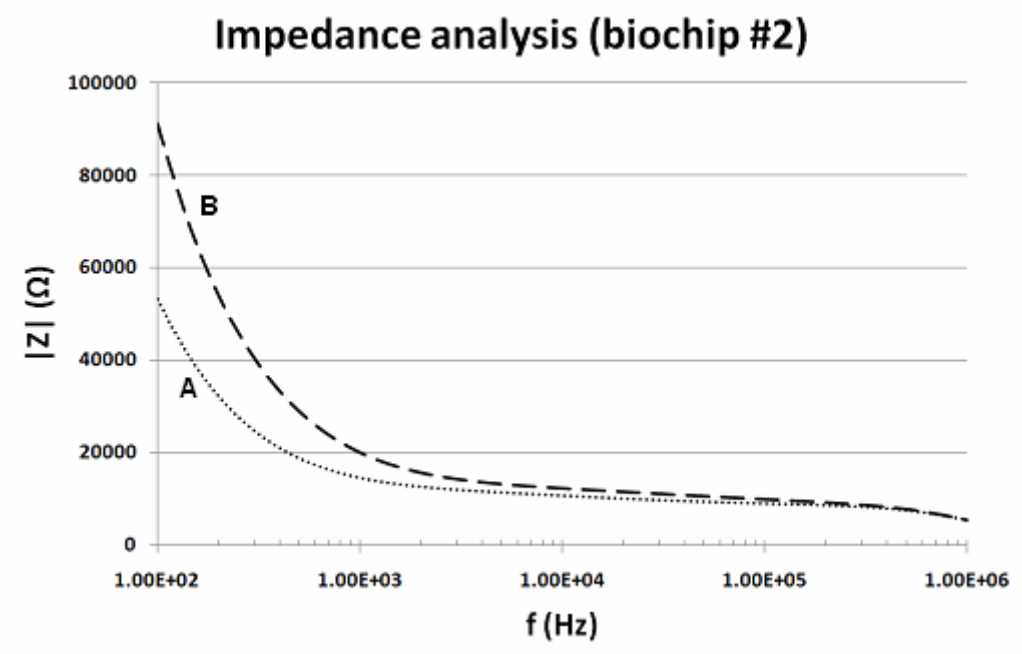

FIGURE 3. Impedance test with medium (A) and A549 cells (B)

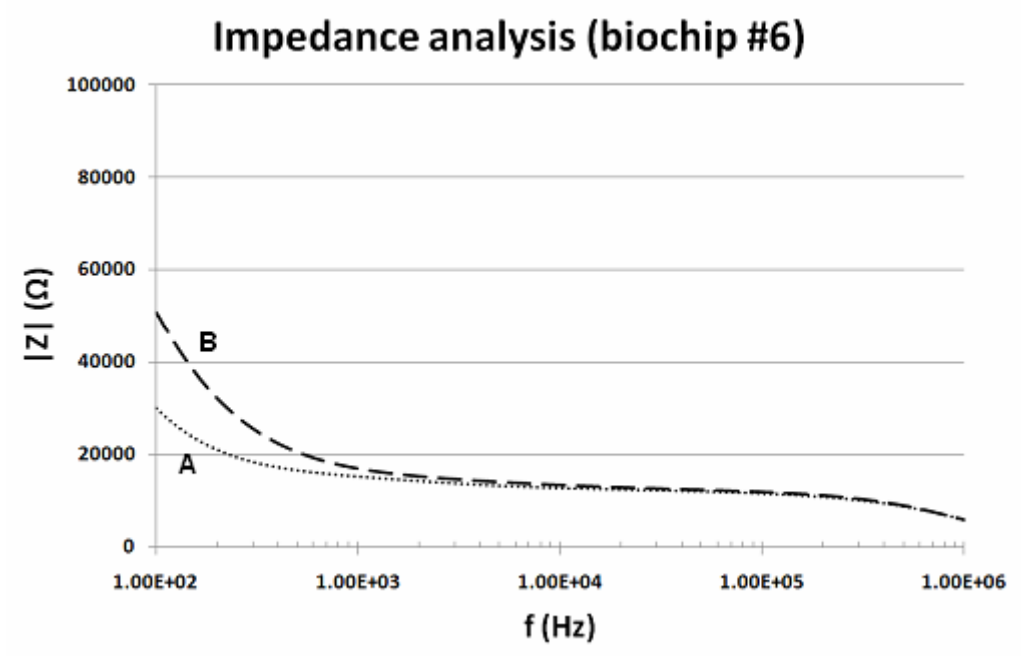

FIGURE 4. Impedance test with medium (A) and A549 cells (B)

Even if the impedance analyses were performed in a wide frequency range, the response of the cells to the current was analysed between 1kHz and 100kHz, as at lower frequencies the cell membranes act as capacitors and the current does not flow through the cells, while at higher frequencies the membranes shield the cells and again no flow of current is allowed through them.

Biochip #2, with a finger width of 60μm and a finger gap of 20μm, showed a gap of 1.6kΩ between the test with the medium and the test with the cells at 10kHz (figure 5), while biochip #6, with a finger width of 40μm and a finger gap of 40μm, showed a gap of 0.65kΩ between the test with the medium and the test with the cells at 10kHz (figure 6). These

preliminary results highlight the possibility to increase the sensitivity of the biochips to the cells response by changing the electrode configuration.

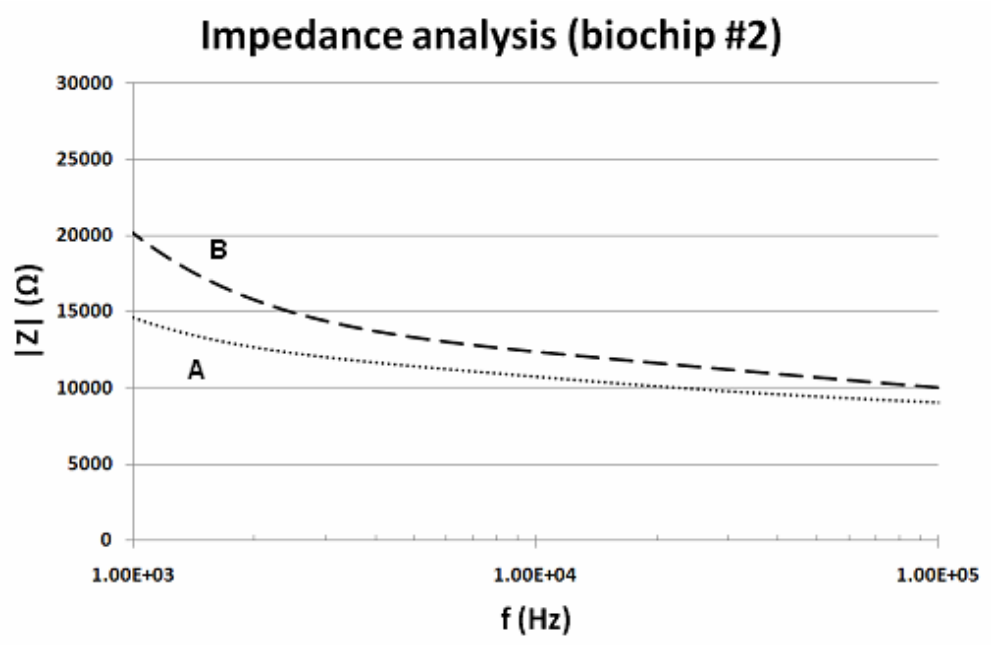

FIGURE 5. Impedance test with medium (A) and A549 cells (B) between 1kHz and 100kHz

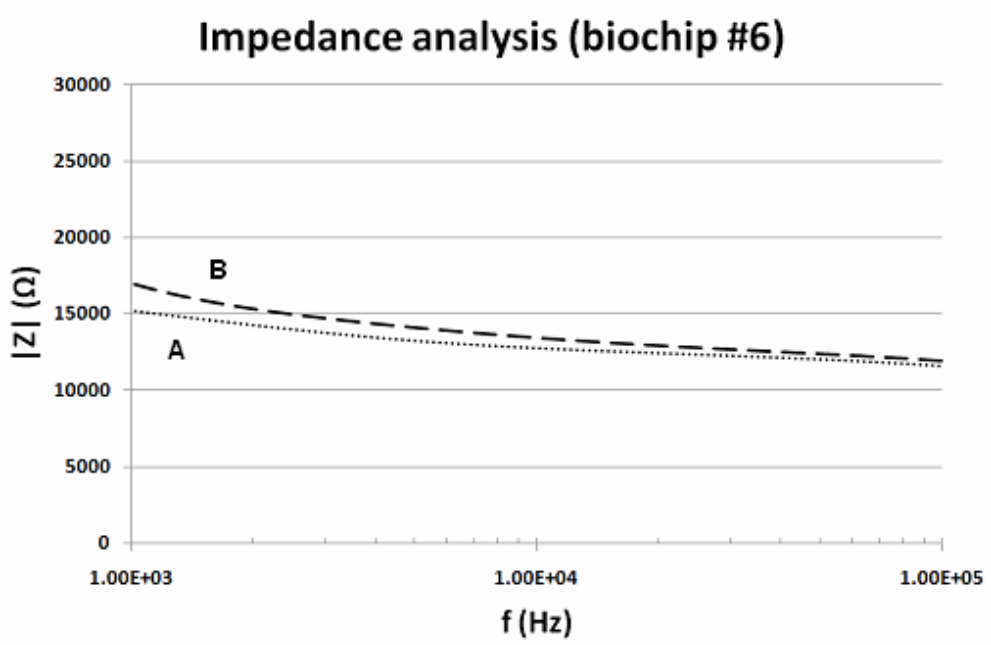

FIGURE 6. Impedance test with medium (A) and A549 cells (B) between 1kHz and 100kHz

As the ITO deposited on the Pyrex wafer is a transparent material, it was possible to verify the confluence of the layer of the A549 cells on the biochip surface. Figure 7A shows an image acquired using the reverse microscope module, while Figure 7B shows an image acquired using the inverted phase contrast microscope.

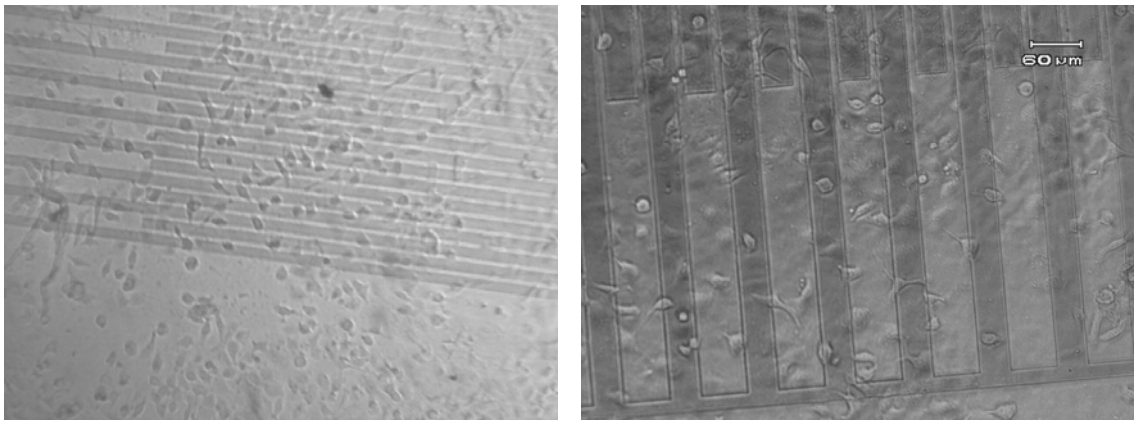

FIGURE 7. Images acquired with reverse microscope module (7A) and inverted phase contrast microscope (7B)

CONCLUSION

The results presented in this article highlight how the configuration of an ITO electrode for impedance measurement on cell cultures is more sensitive when there is a small finger gap and a big finger width, as in the case of biochip #2 (gap 20µm and width 60 µm) and biochip #6 (gap 40µm and width 40 µm).

Future tests will be performed using all the nine configurations in order to identify the optimal one for the impedance analyses using A549 cells.

This technology will cover a wide range of applications, such as screening of potential anti-cancer drugs and tumour invasion, and will be applicable to clinical, biopharmaceutical and environmental monitoring.

ACKNOWLEDGMENTS

The authors acknowledge the Irish Research Council for Science, Engineering and Technology (IRCSET) for funding this research.

REFERENCES

1. Alexander Jr, F., D.T. Price and S. Bhansali. 2010. “Optimization of Interdigitated Electrode (IDE) Arrays for Impedance Based Evaluation of Hs 578T Cancer Cells”. *Journal of Physics: Conference Series.* 224 (2010) 012134.
2. Asphahani, F. and M. Zhang. 2007. “Cellular impedance biosensors for drug screening and toxin detection”. *Analyst.* 132: 835-841.
3. Choi, C.K., A.E. English, S. Jun, K.D. Kihm and P.D. Rack. 2007. “An endothelial cell compatible biosensor fabricated using optically thin indium tin oxide silicon nitride electrodes”. *Biosensors and Bioelectronics.* 22 (2007): 2585-2590.
4. Moore, E., O. Rawley, T. Wood and P. Galvin. 2009. “Monitoring of cell growth *in vitro* using biochips packaged with indium tin oxide sensors”. *Sensors and Actuators B.* 139 (2009): 187-193.