Proliferation and Cytotoxicity Monitoring with ECIS®

Electric Cell-substrate Impedance Sensing (ECIS®) is a widely utilized and preferred technology to quantify a variety of cellular phenotypic behaviors including proliferation, viability, barrier function, and migration. Historically, cell viability assays include intrusive labeling techniques and are mostly limited to single timepoint measurements and subjective results. ECIS® solves these issues by monitoring proliferation and cytotoxicity without the use of intrusive labeling and the quantified data is taken continuously in real-time while the cells remain incubated

Introduction

Measuring cytotoxicity in vitro involves many complicated procedures and typically requires intrusive labeling techniques such as dyes, fluorescence, luminescence, and more. Not only are these procedures difficult to implement, many offer only endpoint measurements, discounting the importance of time course changes of cell behavior following the addition of potential toxicants. Electric Cell-substrate Impedance Sensing (ECIS®) instrumentation allows users to monitor cytotoxic effects of cells in vitro continuously in real-time, without labels, and all while under tissue culture incubation.

ECIS® is a label-free method to measure many cell behaviors including cell proliferation, cytotoxicity, ECM attachment, wound-healing migration, junctional barrier function, and more. ECIS® collects these measurements continuously in real-time while the cells remain incubated by using a non-invasive alternating current sent through gold electrodes located at the bottom of ECIS® tissue culture wells. As cells grow over these electrodes, the current is impeded by the insulating cell membranes causing a increase in overall impedance. The results of the impedance changes are displayed in graphical format of impedance versus time by ECIS® software.

High AC Frequency for Proliferation and Cytotoxicity Assays

The overall electrical impedance changes in ECIS result from variations in resistance and capacitive reactance (the reactance depends inversely on the capacitance as well as the applied AC frequency). ECIS® technology isolates these two factors by complex impedance measurements, where the choice of AC frequency will determine which factor contributes more to the total impedance (Giaever & Keese, 1991).
For example, when measuring cellular impedance at low AC frequency (e.g., < 4,000 Hz), the membrane impedance (capacitive reactance) is high, and most of the current now flows resistively under the cells and

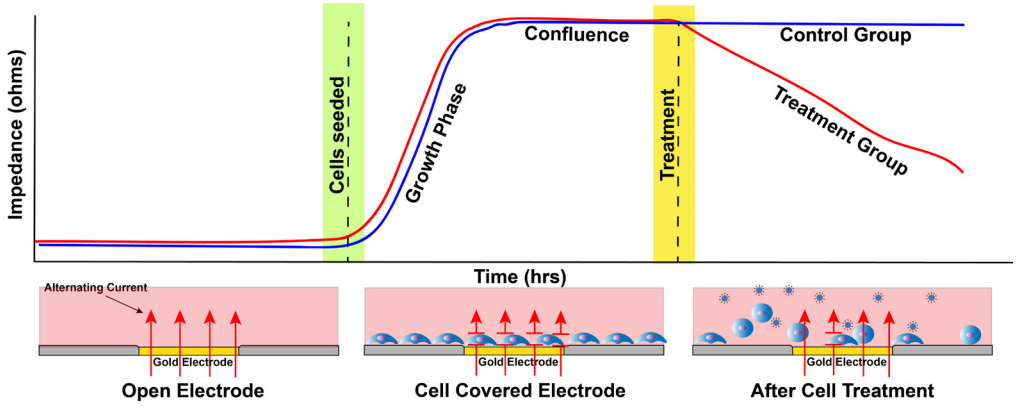

Figure 1: Schematic representation of ECIS data with impedance vs time. As cells grow and cover electrodes, impedance data rises proportional to cell coverage of the gold electrodes.

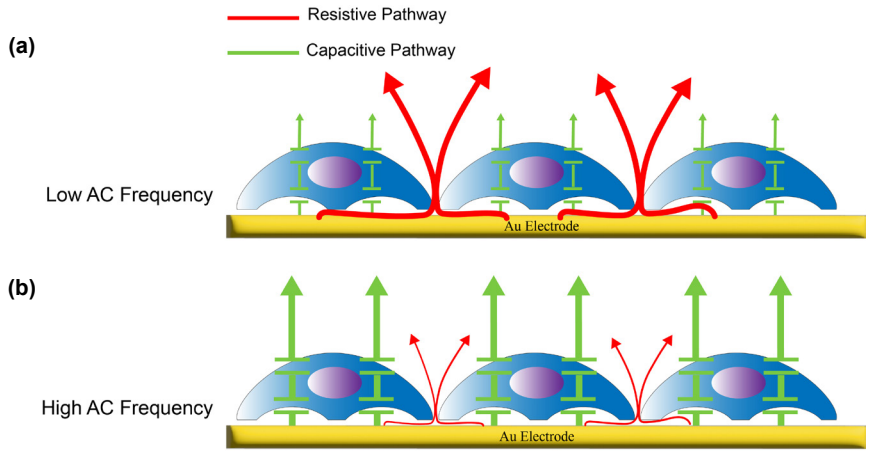

Figure 2: Sources of impedance of alternating current paths through cell monolayers. **(a) At** low AC frequency, the majority of impedance is from resistance due to the current flowing around the paracellular space (red arrows), whereas with **(b)** At high AC frequency, the majority of impedance is due to the current capacitively coupling through the cell membranes (green arrows)

through the tight spaces of the cellular junctions (Figure 2a). At high AC frequencies (e.g., > 32,000 Hz), on the other hand, the capacitive reactance of the cellular membrane is relatively small, and the majority of the current capacitively couples through the insulating cell membranes with little current passing through the paracellular path (Figure 2b). By exploiting these differences, ECIS® has the ability to utilize capacitance measured at high AC frequency for quantifying cellular substrate coverage in terms of growth and cytotoxicity. When cells attach and spread over the electrodes at high AC frequency, the capacitance decreases in a linear manner with the proportion of cell-substrate coverage and will bottom out upon confluence (see Figure 3). When cells experience toxicity and even death, morphological changes will occur, and the cells will detach from the electrodes, resulting in capacitance increases and impedance drops.

Quantifying Cell Coverage Continuously

Cell viability assays such as colorimetric or fluorescent assays pose potential problems such as being cytotoxic themselves or have strict time sensitivity for accurate measurements. These assays also pose the risk of interference with the compound being used as the independent variable. ECIS® allows users to measure proliferation and/or cytotoxicity continuously in real-time by quantifying the viability of the cells while they remain under incubated conditions. And since the cell behaviors are being measured by the currents, there is no need for intrusive labeling techniques such as fluorescence or dyes.

Figure 3 shows the time range of MDCK I cells from when they were freshly seeded at time 0 at varying quantities until they attached or proliferated to confluence. As the data shows, the highest seeding quantity (250,000/well) reaches a plateau at ~3 hours. This seeding density was high enough that there was no need for proliferation due to the cells attaching and immediately reaching confluence, whereas the lowest seeding density (5,000/well) reaches a confluent plateau at ~75 hours (Figure 3), requiring proliferation to reach confluence. Since we're observing attachment and proliferation of the cells, we're using high frequency capacitance at 64,000 Hz to measure cell coverage. Notice as the cells attach and proliferate, the capacitance values drop in nanofarads. This is because the open gold-film electrode (without cells) has a relatively high specific capacitance of ~10 microfarad/cm^2. As the cells attach and spread upon the gold electrode, their membrane capacitance of ~1 microfarad/cm^2 is in series with the electrode capacitance. This results in a *drop* in the measured capacitance as the cells attach and proliferate, reaching a minimum once the monolayer of cells is confluent.

Not only can the attachment and proliferation of cells be measured using ECIS, the cytotoxicity of cells in terms of cell death can also be measured (Xiao, Lachance, Sunahara, & Luong, 2002). When cells experience necrosis from an added toxic substance, they will detach from the electrodes.

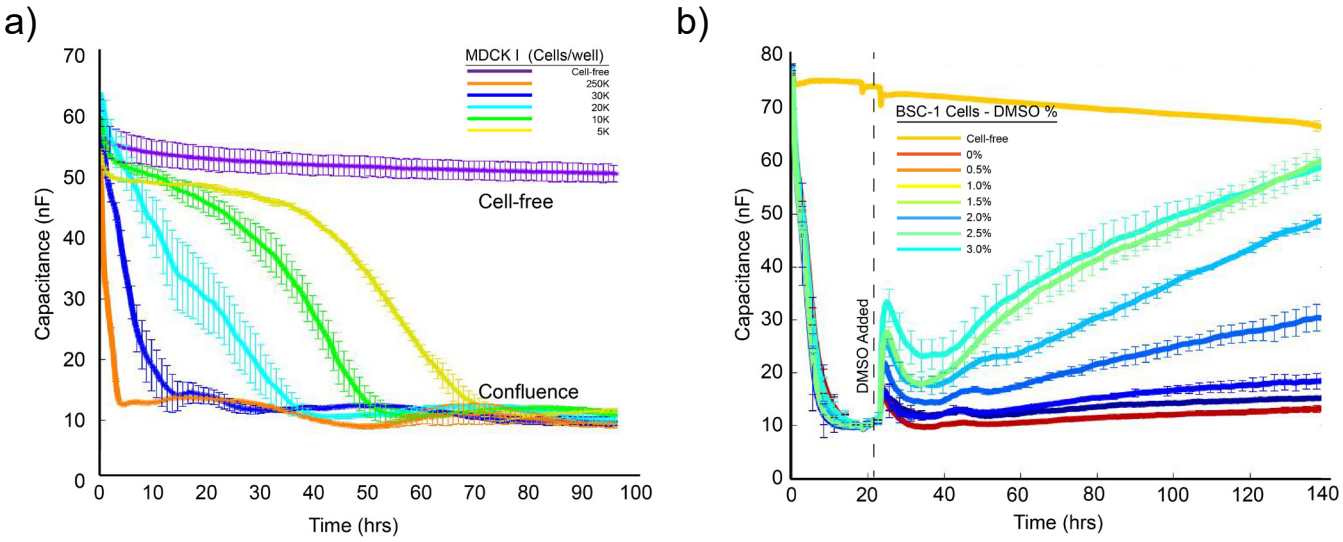

Figure 3: Measuring cell coverage using high frequency capacitance. a) Proliferation rates to confluence of MDCK I cells seeded at varying quantities per well ranging from 5K (yellow) to 250K (orange) per well. b) Toxicity curves of BSC-1 cells treated with varying concentrations of dimethyl sulfoxide ranging from 0% (red) to 3% (turquoise).

As the cells detach, the capacitance values will rise towards the cell-free capacitance value in proportion to the reductions of the percentage of cells covering the electrodes. This is displayed in Figure 4 where BSC-1 cells were seeded and grown to confluence and were then treated with dimethyl sulfoxide (DMSO) at ranging concentrations. Notice that the highest concentration of 3% DMSO is rising towards the cell-free value, and the lowest concentration of 0.5% remains close to the normal confluence of the cells that received no DMSO (Figure 4), suggesting very little toxicity to the cells at that concentration.

Conclusion

Electric Cell-substrate Impedance Sensing is a well established, label-free method of measuring cell behaviors in vitro. Most traditional cell viability assays require intrusive labeling techniques and only provide single endpoint results. By harnessing high frequency alternating currents, ECIS® monitors cell attachment, growth, and cytotoxicity by following the capacitance changes of electrodes used as substrates for cell growth. Not only is there no need for labeling, ECIS® allows users to monitor cell behaviors continuously in real-time while the cell cultures remain incubated, providing quantified data of the entire experiment.

Giaever, I., & Keese, C. R. (1991). Micromotion of mammalian cells measured electrically. Proceedings of the National Academy of Sciences of the United States of America, 88(17), 7896–7900. https://doi.org/10.1073/pnas.88.17.7896

Xiao, C., Lachance, B., Sunahara, G., & Luong, J. H. T. (2002). Assessment of cytotoxicity using electric cell-substrate impedance sensing: concentration and time response function approach. Analytical Chemistry, 74(22), 5748–5753. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12463358