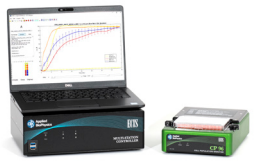
Measuring Cytotoxicity with the ECIS® CP96 Cell Population Monitoring System



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, cytotoxicity assays include intrusive labeling techniques and are mostly limited to single timepoint measurements and subjective results. Applied BioPhysics now offers the ECIS® CP96, which utilizes the technology of ECIS® to monitor cytotoxic effects continuously in real-time, and label-free.

Cytotoxicity In Vitro

Measuring cytotoxocity 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 [1]. 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 unique method to quantitatively measure cell behaviors in vitro by sending weak alternating current (AC) between gold electrodes acting as substrates for cell attachment and growth. The subsequent voltage changes caused by the impedance of the cellular coverage of the electrodes are collected and converted into graphical format. The overall electrical impedance changes are determined by two factors: capacitance and resistance. The ECIS® technology takes advantage of these factors by isolating them using complex impedance measurements, where the choice of AC frequency will determine which factor contributes more to the total impedance [2,3]. For example, when measuring cellular impedance at high AC frequency (e.g. 48 kHz), the electrical current tends towards capacitively coupling through the cell membrane as opposed to passing through the paracellular space which occurs at lower AC frequencies (e.g. 4000 Hz) (figure 1). 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 capacitive part of the impedance grows in linear proportion to the cell-substrate coverage and will plateau upon confluence. When cells experience toxicity and even death, morphological changes will occur and the cells will detach from the electrodes causing this impedance to drop with the same proportionality.

ECIS® CP96

Applied Biophysics now offers the ECIS® CP96 instrument for quantifying cytotoxicity in tissue culture. This complete turn-key system provides users the ability to reproducibly measure the rate of cell growth and death using high AC frequency without the need for intrusive labeling. These data can be collected for hours, days or even weeks at a time. Data is displayed in graphical format by the ECIS® software as changes in CellX over time, where CellX refers to the percentage of coverage from a cell monolayer (hence values from 0 to 100).

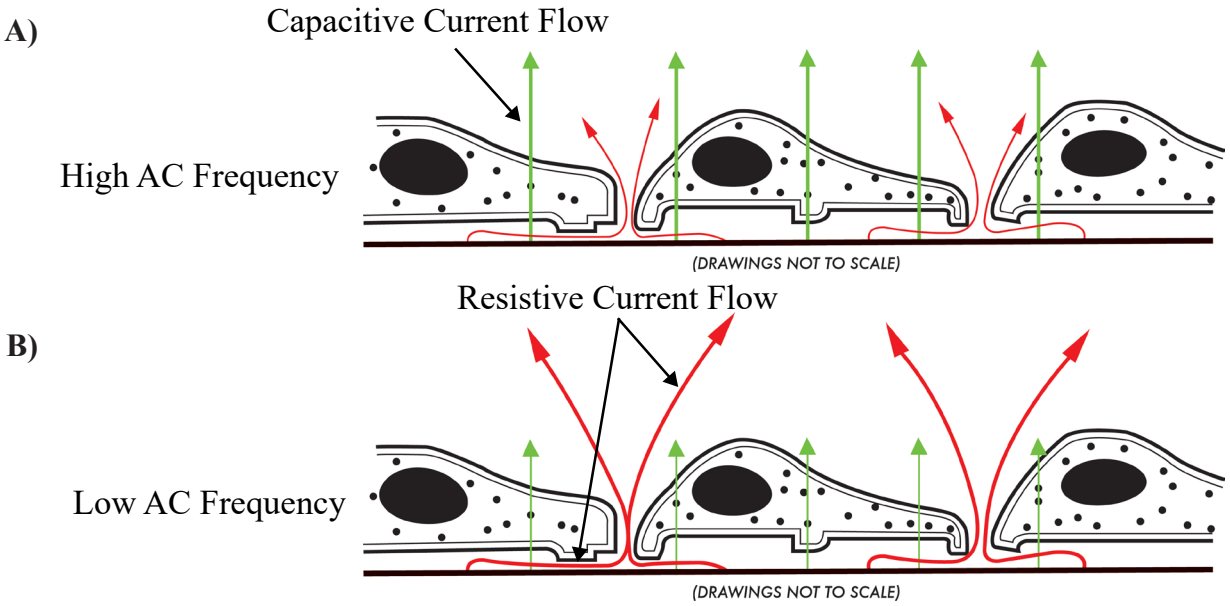


Figure 1: Diagram representing effects of impedance dispersement on cell layers with differing frequencies of alternating current. A) At high AC frequency, the contribution to total impedance is largely from capacitive coupling through the cell membranes (green arrows), B) whereas with low AC frequency the majority of impedance is due to attachment and barrier resistance in the paracellular space (red arrows).

Once the cells experience deleterious effects following applied treatments, ECIS® software can then auto-calculate EC50 values using the "four parameter logistic curve" which includes minimum and maximum values, inflection point (EC50), and the slope of the inflection point termed the Hill Coefficient. An example of this is displayed in the figure below. BSC-1 cells were treated with varying concentrations of DMSO and the EC50 value was calculated (Figure 2). In the early portion of the impedance time course, the CellX data rises from 0 up to 100, representing growth of the cells to a confluent layer. Upon reaching confluence, cells were treated with increasing concentrations of DMSO and cytotoxicity was subsequently monitored. As the data clearly shows, with increasing concentrations of DMSO the cytotoxic effects also increase. The boxes below the graph show the EC50 sigmoidal graph and the parameters that were applied. According to the calculation, the EC50 of the DMSO treatment resides at 1.72%.

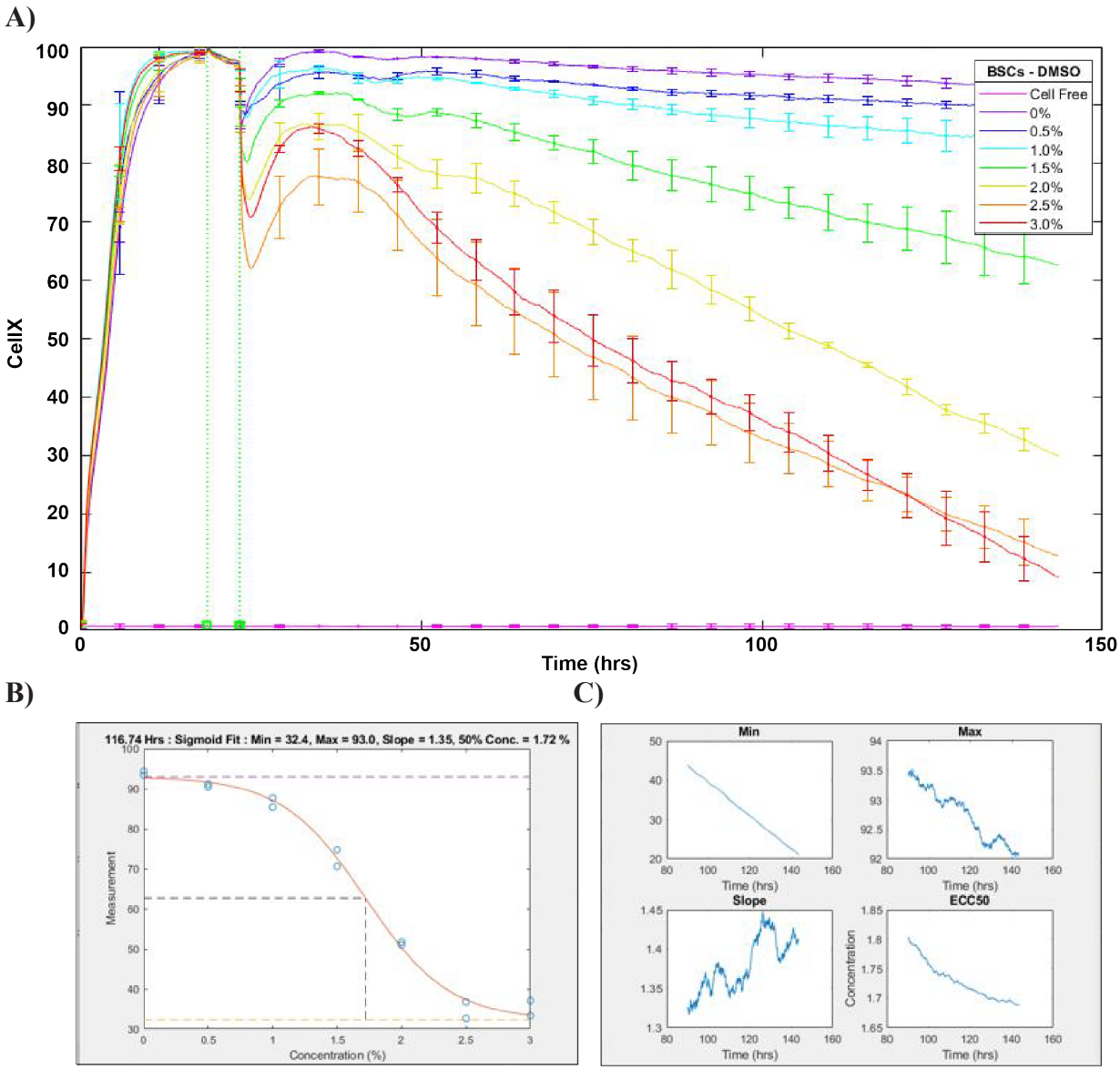


Figure 2: BSC-1 cells treated with varying concentrations of DMSO. A) Graphical representation of cell coverage from 0 to 100% CellX. B) EC50 Sigmoidal curve of DMSO concentrations. C) The parameters of the "four parameter logistic curve."

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