ECIS® Electroporation and Monitoring

Gaining entry of membrane-impermeable molecules into cells in culture requires methods that bypass the chemical or physical restraints offered by the cell membranes. Once accomplished, these methods subsequently need verification to confirm the process has taken place. ECIS® instrumentation offers a unique opportunity to introduce membrane-impermeable compounds into cells growing upon the ECIS® electrodes via ECIS® automated electroporation and to subsequently monitor the cell morphological changes due to the compound's introduction.

Introduction

Introducing membrane-impermeable molecules (MIM's) into cultured cells is an important aspect of cell biology research. This process allows researchers to investigate the role of compounds normally blocked from externally entering the cell, contributing to a better understanding of cell function, disease mechanisms, and potential therapeutic targets. MIMs include significant macromolecules such as DNA and RNA, enzymes, and antibodies, as well as large constructs such as nanoparticles. In addition, many low molecular weight compounds cannot cross the fatty plasma membrane due to their chemical/physical nature. Typically, the methods that facilitate the entry of MIM's require confirmation that the introduction of the molecules of interest has, in fact, occurred. This confirmation is often in the form of labeling techniques such as fluorescence alongside the desired MIMs.

To study the effect of membrane-impermeable compounds, biologists have devised various means to facilitate their entry, including liposomes, cell-penetrating peptides, and microinjection; the method we will be considering here is electroporation. This technique involves the application of a high electric field across the cell membrane, resulting in a temporary breakdown of the insulating membrane with the formation of pores sufficiently large to allow the entry of MIMs into the cytoplasm (Figure 1). The strength of the required electric field (V/m) depends both upon the applied voltage and the distance between the electrodes.

Electric Cell-substrate Impedance Sensing (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 impedance measurements continuously in real time while the cells remain incubated. This is accomplished using a weak, non-invasive alternating electrical current sent through gold electrodes at the bottom of ECIS® tissue culture array wells. As cells grow over these electrodes, the insulating cell membranes impede the current, and results are reported in graphical format.

Due to the relatively close proximity of the electrodes in the ECIS® arrays, currents resulting in small voltage drops are sufficient to produce the high electric field required for pore formation, and so, unlike most electroporation devices, there is no danger of electric shock.

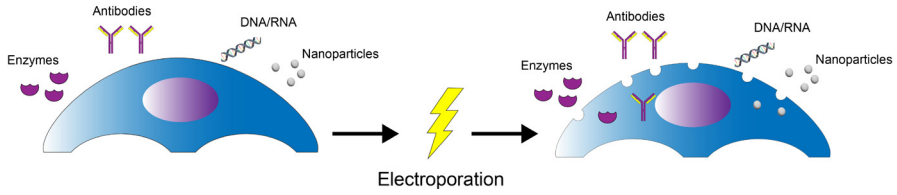

Figure 1: Model representation of transfection of membrane-impermeable molecules via electroporation.

ECIS® has been demonstrated to detect changes in cell behaviors due to various external molecules that either bind to receptors on the cell surface or can directly pass through the plasma membrane and enter the cell. However, in this normal mode of operation, the instrument cannot detect the response of cells to compounds that do not bind to cell receptors or are otherwise unable to enter the cytoplasm of the cell.

Cell Real-time Electroporation and Monitoring (CREAM) Assay

The Cell Real-time Electroporation and Monitoring (CREAM) assay uses the ECIS® electronics and arrays in two very distinct operations to study the effect of MIMs upon cell behavior.

1. An electroporation pulse that invasively causes electroporation of the cell membranes
2. A standard ECIS® non-invasive current mode to monitor the cell response.

The figure below outlines these two operations (Figure 2).

The graph shows the time course change of the normalized impedance of two wells with confluent cell monolayers measured at 4 kHz using non-invasive levels of current (1µA). In the experimental well, there is a cytotoxic drug dissolved in the medium.

The measurement is briefly stopped ~1.5 hours into the run, and both wells then receive a high current pulse (~1 mA at 40k Hz for 200 milliseconds), resulting in the electroporation of the plasma membranes. This pulse is followed by a return to the previous non-invasive current to study the fate of the cells.

Whereas the control well returns to the starting impedance shortly after the electroporation, the experimental well shows a steadily decreasing impedance drop due to the introduction of the membrane-impermeable cytotoxic compound into the cells.

There are some features in the graph to consider.

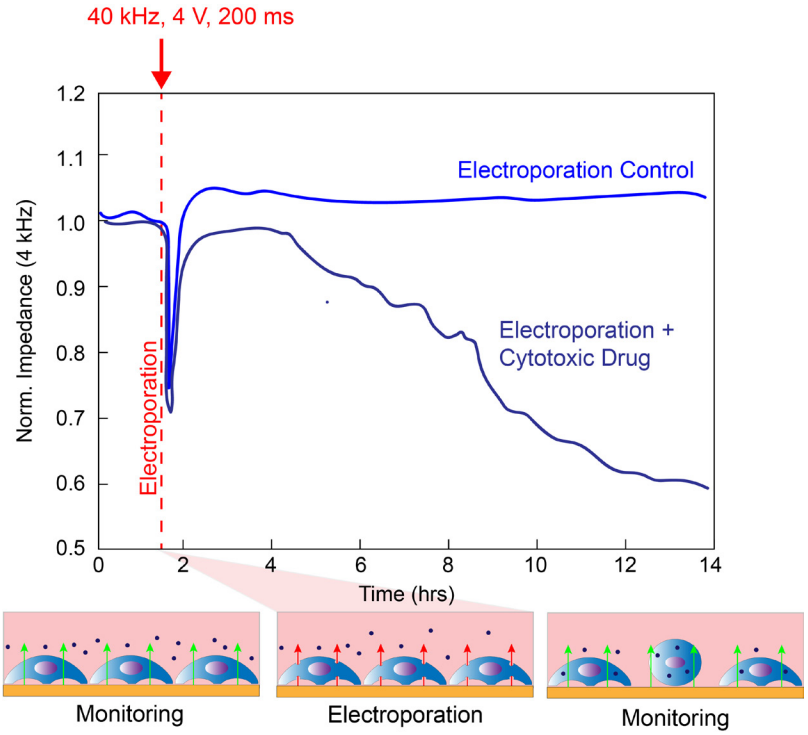

Figure 2: Normalized impedance data before and after ECIS automated electroporation of NRK cells treated with cytotoxic MIMs against a vehicle control. Data courtesy of Stolwijk et al., 2011.

- The cytotoxic compound is present in the experimental well's medium at time zero. Still, due to its membrane-impermeable nature, there is no effect on the cells, and both confluent layers appear identical before electroporation.
- The control well with medium without the cytotoxic compound is also electroporated and shows a response to the treatment. This behavior is commonly observed as the electroporation results in some mixing of the cytoplasm and the extracellular environment. Not unexpectedly, this affects cell morphology and, hence, the ECIS® measurement. The effect is short- lived – approximately 30 minutes.
- The current used for electroporation is nearly one thousand-fold higher than that used for ECIS® monitoring to produce the high field required for membrane poration*. Notice, this current is applied at a relatively high frequency (40,000 Hz), which is essential for the assay to succeed. Due to the nature of the gold electrodes, at lower frequency, say 4,000 Hz, most of the voltage drop is across the electrode interface, rather than the cell layer. This can severely damage the ECIS® electrodes, effectively ending the measurements. At the high frequency, most of the voltage drop is now across the cell layer rather than the electrode interface – protecting the electrode and causing the desired cell membrane breakdown.

  *(Note: Due to the use of electrodes as cell substrates, the mA currents used for pore formation produce the required high electric fields with only a few volts. So, unlike most electroporation devices, there is no danger of electric shock using ECIS®)

Below are the results of a CREAM assay with NRK cells treated with sodium azide (Figure 3a) and bleomycin (Figure 3b), both membrane-impermeable compounds (Stolwijk et al., 2011). As shown in the figures, the data following electroporation (arrows) shows a decrease in impedance corresponding with the concentrations of the added impermeable compounds. Notice that the control wells that were electroporated but had no toxic compounds (Fig 3b) had an initial drop from the electroporation itself but quickly returned to baseline levels, whereas the wells with no electroporation but did have the compound show no drop in impedance.

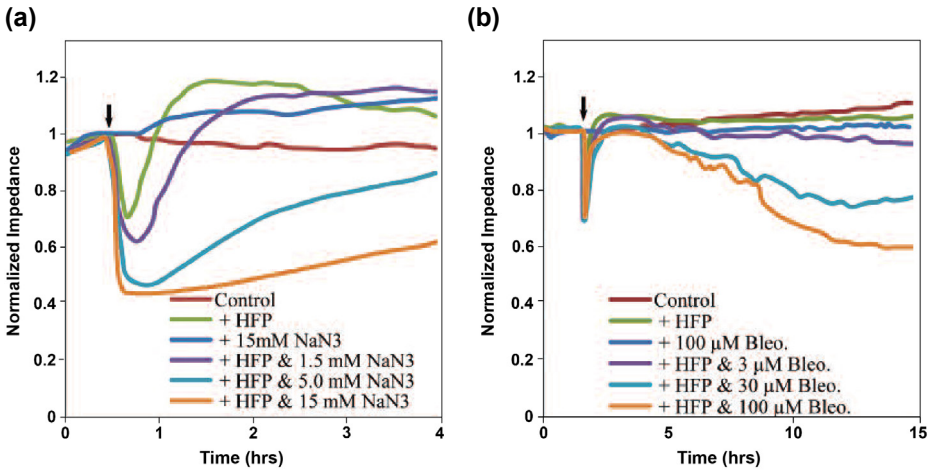

Figure 3: Cellular response to the introduction of toxins. (a) Time course of the normalized impedance at a sampling frequency of 4 kHz before and after electroporation(arrow) (HFP) of NRK cells incubated with different concentrations of sodium azide. Control cells were incubated with15 mM sodium azide but were not electroporated or remained untreated. (b) Time course of the normalized impedance upon electroporation (arrow) (HFP) of NRK cells with different concentrations of bleomycin. Control cells were incubated with 100 µM Bleomycin but were not electroporated or they remained entirely untreated (Stolwijk et al., 2011).

Conclusion

Entry of membrane-impermeable molecules into the cytoplasm of cells can be accomplished by ways of physical or chemical means. Electroporation is a method to facilitate entry of MIMs into the cytoplasm that uses electrical fields to create pores in the cellular membrane large enough to allow entry of most impermeable compounds. ECIS® not only offers the capability to electroporate cell membranes for transfections purposes, but also quantitatively monitors the cell morphology and behaviors continuously in real-time following the entry of the compounds into the cytoplasm. These abilities make ECIS® an ideal method for transfection assays.

Stolwijk, J. A., Hartmann, C., Balani, P., Albermann, S., Keese, C. R., Giaever, I., & Wegener, J. (2011). Impedance analysis of adherent cells after in situ electroporation: non-invasive monitoring during intracellular manipulations. Biosensors & Bioelectronics, 26(12), 4720–4727. https://doi.org/10.1016/j.bios.2011.05.033