Measuring TEER with the ECIS® 8-well Transfilter Array

Transepithelial electrical resistance (TEER) has been a widely utilized method to quantitatively monitor cell barrier function of epithelial and endothelial monolayers. Typical measuring methods using TEER traditionally consisted of single timepoint measurements in non-tissue-culture environments, often with variable results. Applied BioPhysics Inc. has devised the ECIS® 8-well Transfilter Array for the ECIS® Z-theta instrumentation. With this adapter, one can conduct TEER measurements on cell-culture permeable inserts continuously, in real-time, and under tissue-culture incubation with fixed position electrodes for highly repeatable results.

Introduction

The barrier function of cell monolayers has been an important characteristic and metric to understanding the dynamics and health of epithelial and endothelial cells. By studying the barrier function caused by the cell-cell junctional complexes of monolayers, researchers can gain a greater understanding of the role of these tissues in homeostasis and diseases, as well as the substances that modify them. One popular and label-free method to measure the permeability of cell monolayers is transepithelial electrical resistance (TEER).

In TEER measurements, a weak alternating electrical current (AC) is passed between two electrodes flanking a cell monolayer on a culture transwell insert. TEER is defined by the measured cell resistance to current flow (ohms) multiplied by the cell-substrate area (cm^2). Until recently, TEER measurements had many limitations, including single-point measurements, noncanonical environmental conditions (outside tissue-culture incubation), and cumbersome electrode positioning.

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 and reported in graphical format (Figure 1).

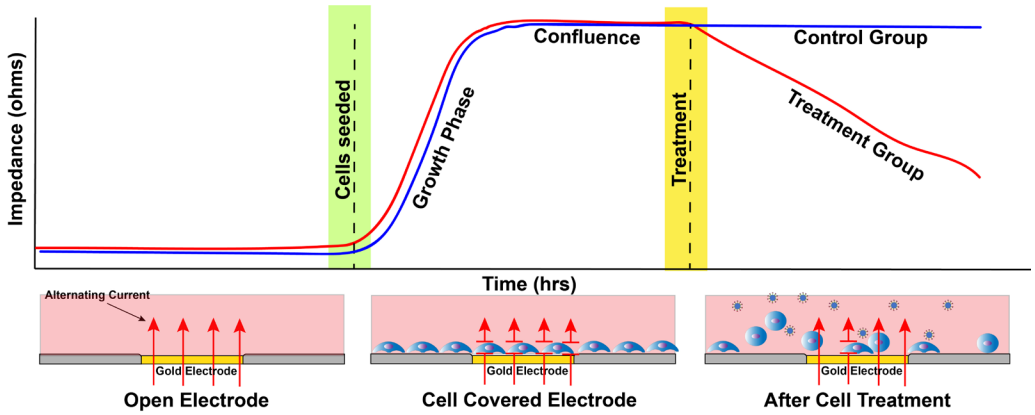

Figure 1: Schematic representation of ECIS® data with impedance vs time. As cells grow and cover electrodes, impedance data rises proportional to cell coverage of the gold electrodes.

Low AC Frequency for TEER

Like TEER, the ECIS® measurement uses weak alternating currents to measure impedance as cells are grown upon electrode substrates (Mitra, Keese, & Giaever, 1991). ECIS® monitors complex impedance (both resistance and capacitance) at multiple AC frequencies allowing the user to monitor many cellular phenotypes including barrier function, proliferation, cytotoxicity, and attachment and spreading in real time (Szulcek, Bogaard, & van Nieuw Amerongen, 2014). At high AC frequencies (e.g., 32,000 Hz) the impedance (capacitive reactance) of the cellular membrane is relatively small, and the current mainly *capacitively* couples through the insulating cell membranes with little current passing through the paracellular path (Figure 2a). At low AC frequencies (e.g., < 4,000 Hz) on the other hand, the membrane impedance is high, and most of the current now flows *resistively* under the cells and through the tight spaces of the cellular junctions (Figure 2b). As a result of this difference, low frequency measurements are ideal to measure the barrier function resistance (ohms) and hence TEER (ohms-cm^2).

ECIS® 8-well Transfilter Array

Applied BioPhysics Inc. offers a specialized array for conducting TEER measurements continuously in realtime, and while incubated. The ECIS® 8-well Transfilter Array (8wTFA) uses standard 24-well permeable cell culture inserts and connects with the ECIS® Z-theta electronics using the 16-well array station (Figure 3a). The array is composed of three parts: 1) a semi-disposable baseplate containing gold film addressable electrodes at the bottom of each well in conjunction with 2) a common gold dipping pin electrode array used as the counter electrode and 3) the array lid (Figure 3b).

During data collection with the ECIS® 8wTFA, weak AC current passes between the electrodes at the bottom of each well and the dipping pin electrode insert. As the current passes through the cell monolayer between the gold electrodes, the resulting resistance and capacitive reactance changes caused by the cell barriers and substrate coverage, respectively, are recorded and displayed in graphical format. An example of this is shown in Figure 4, where MDCKII cells were monitored using the ECIS® Z-theta with the 8wTFA. As the data implies, the MDCKII cells reached confluence at roughly 10 hours post-inoculation where the capacitance bottoms out (Figure 4b). In contrast, the TEER levels begin to rise just prior to confluence and continue to rise until a peak and then a plateau is reached (Figure 4a), suggesting the formation of a mature cell monolayer exhibiting barrier formation. While the adhesion and spreading of cells does restrict the electrical current, notice that the majority of the TEER increase follows the establishment of a fully confluent layer, suggesting that this increase is largely due to paracellular junctions of the cell barriers.

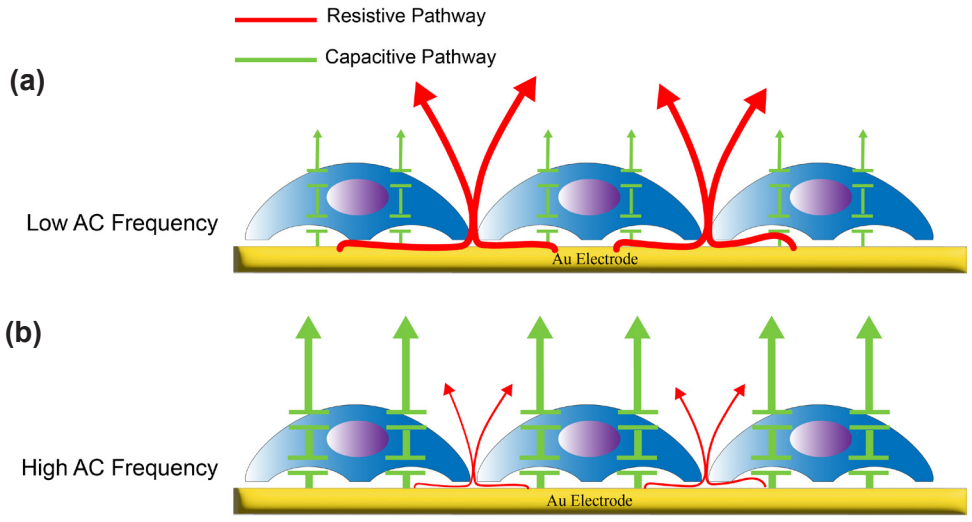

Figure 2: Sources of impedance of alternating current paths through cell monolayers. **(a)** At high AC frequency, the majority of impedance is due to the current capacitively coupling through the cell membranes (green arrows), whereas with **(b)** low AC frequency, the majority of impedance is from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

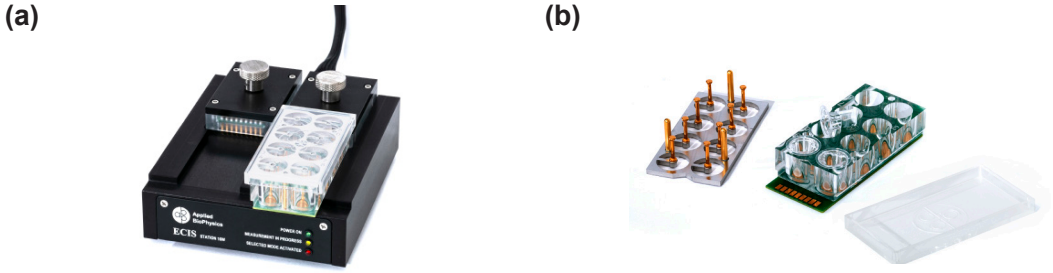

Figure 3: **(a)** The assembled 8WTFA fits into the ECIS Z-theta 16-well array station. **(b)** ECIS® 8-well Transfilter Array is comprised of a dipping pin common electrode (plated gold), a baseplate with addressable gold film electrodes at the bottom of each well and a lid.

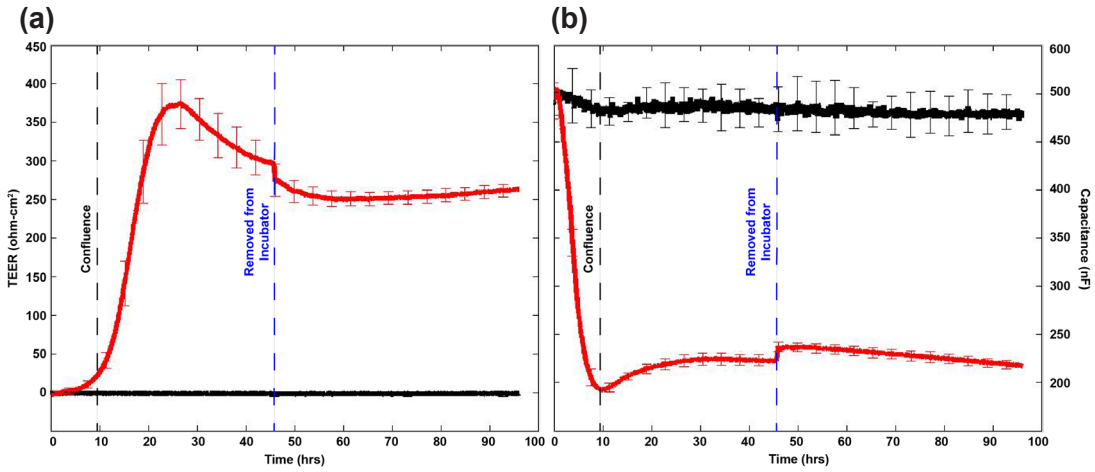

Figure 4: MDCK II cells (red) vs cell-free filters (black) grown in the ECIS® 8-well Transfilter Array Adapter and monitored with the ECIS® Z-theta. **(a)** TEER measurements are continuously recorded at low AC frequency (125 Hz) while **(b)** simultaneously recording capacitance for cell growth to confluence at high AC frequency (64 kHz).

Mitra, P., Keese, C. R., & Giaever, I. (1991). Electric measurements can be used to monitor the attachment and spreading of cells in tissue culture. Biotechniques, 11(4), 504–510.

Szulcek, R., Bogaard, H. J., & van Nieuw Amerongen, G. P. (2014). Electric cell-substrate impedance sensing for the quantification of endothelial proliferation, barrier function, and motility. Journal of Visualized Experiments, (85), 1–12. https://doi.org/10.3791/51300