Quantification of Cell Monolayer Barrier Function with ECIS®

Barrier function assays of epithelial and endothelial cell monolayers have traditionally consisted of labeling techniques such as fluorescence or permeability assays that offer only single point in time measurements. Electric Cell-substrate Impedance Sensing (ECIS®) is a real-time, impedance-based method to study many of the activities of cells when grown in tissue culture without the use of intrusive labeling techniques. In this application note, we will discuss using ECIS to measure the barrier function of cell monolayers by taking advantage of multiple AC frequencies.

Introduction

The barrier function of cell-cell junctions of epithelial and endothelial cell monolayers is important in biological functions not only by separating tissues, but by also allowing selective permeation of various molecules into and out of the underlying tissues. Depending on the physiological location of these monolayers, the barrier integrity varies in function and degree of permeability. Irregularities in barrier function of tissues are associated with multiple types of diseases including respiratory illnesses (Guillot et al., 2013), gastrointestinal complications (Oshima & Miwa, 2016), and even disruption of vascular walls in the blood-brain-barrier correlating to Alzheimer's disease (Chakraborty, de Wit, van der Flier, & de Vries, 2017). Many methods have been employed to measure the barrier function of cell monolayers in vitro, but can be limited to single timepoint measurements and can be difficult to quantify.

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 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).

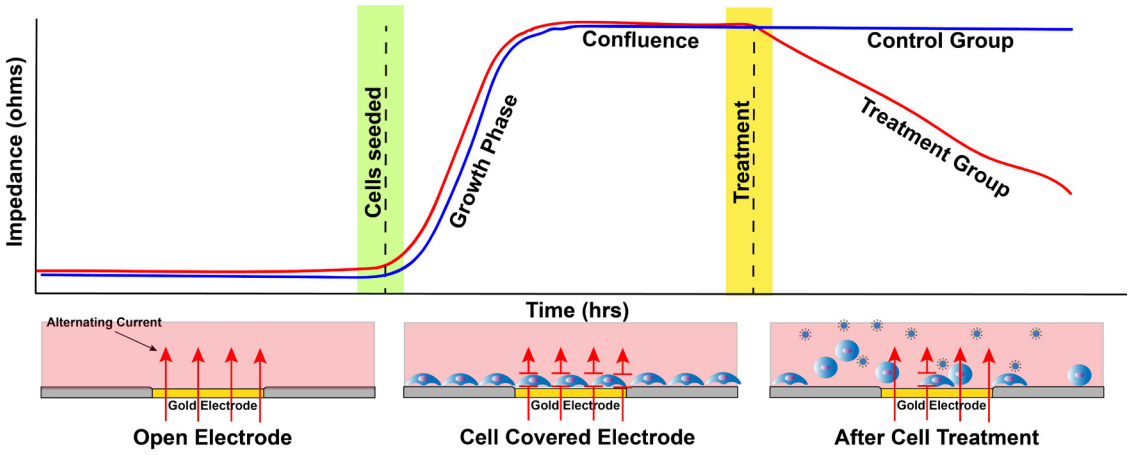

Figure 1: Schematic representation of ECIS data with impedance vs time. As cells grow and cover electrodes, impedance data rises proportional to cell coverage of the gold electrodes.

Low AC Frequency for Barrier Function Assays

Most epithelial and endothelial cell types can be cultured in vitro to form confluent monolayers where it is possible to measure the barrier function afforded by these cell layers. In addition, with the right tools, dynamic changes of the layers can be followed when the cellular environment is altered by exposure to compounds or physical changes such as shear stress.

Electrical currents will always follow the path of least resistance. ECIS® uses an alternating electrical current (AC) with multiple frequencies to measure cellular behaviors when they are attached to the gold film substrates of ECIS® culture wells. At high AC frequencies (e.g., 32,000 Hz) the impedance (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 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 (the solution path) (Figure 2b). As a result of this difference, low frequency measurements are ideal to measure the barrier function and permeability of cell monolayers.

The following data (Figure 3) reports the changes of impedance in two duplicate wells following inoculation of MDCK II cells. The micrographs show that the cell layer is in place and confluent about 3 hours after inoculation, and this is conveyed by the plateau in the impedance at 40,000 Hz. Measuring the same wells at 400 Hz we see the formation of the barrier function is not completed until about 10 hours after inoculation indicated by the plateauing impedance. This is also confirmed microscopically using stains for the junctional proteins E-cadherin and ZO-1 (zona occludens protein) (Data courtesy of Professor Joachim Wegener, Univ. of Regensburg). For tight epithelial cells, the impedance (mainly resistance) at low AC frequencies provides a very effective measure of the layer's barrier function.

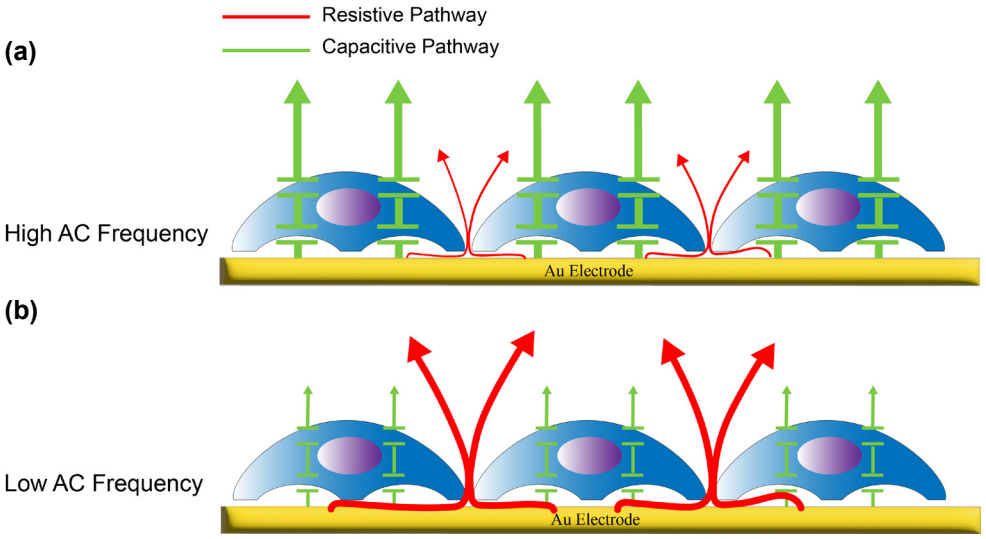

Figure 2: Sources of impedance of alternating current paths through cell monolayers. **(a)** At high AC frequency, the majority of impedance is due to the current capacitively coupling through the cell membranes (green arrows), whereas with **(b)** low AC frequency, the majority of impedance is from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

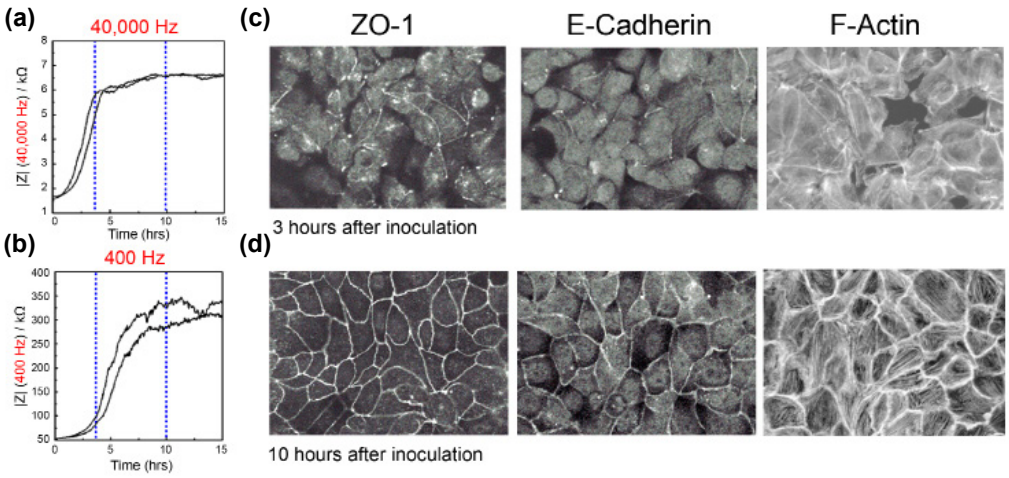

Figure 3: Comparison of confluence and barrier formation of MDCK II cells. **(a)** ECIS® readings at high AC frequency (40,000 Hz) reveals plateauing impedance at ~3 hours suggesting confluence of the monolayer versus **(b)** low AC frequency showing plateauing at ~10 hours suggesting maturation of cell-cell junctional formation. **(c)** Fluorescence images of the same seeding parameters as the ECIS® measurements for zona occludens, E-cadherin, and F-actin 3 hours after inoculation and **(d)** 10 hours after inoculation. Graphs and figures courtesy of Joachim Wegener, University of Regensburg.

Quantifying Barrier Function Continuously

Traditionally, monitoring the barrier formation and permeability of epithelial or endothelial monolayers typically requires fluorescently labeling barrier proteins or conducting permeability assays measuring the flux of molecules that permeate through the monolayers. These assays can be restrictive since the data is collected in single timepoint measurements leaving the researcher ambiguous to the appropriate time point for collection of such measurements following treatments. With ECIS®, the barrier function of cell monolayers is monitored without the use of labeling and is quantified continuously in real-time while the cells remain incubated. To highlight this point, the barrier resistance of MDCK 1 cells were continuously monitored at 250 Hz from initial seeding to barrier maturation as indicated by the plateau in resistance. Once confirmation of the barrier maturation was established, the cells were treated with the known tight junction disrupter cytochalasin D (Stevenson & Begg, 1994) , causing a drop in the low frequency resistance relevant to the increasing concentrations of the treatment (Figure 4).

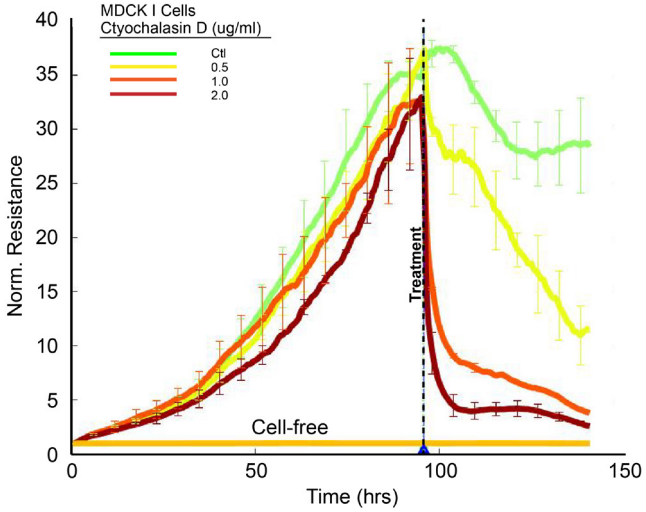

Figure 4: ECIS Measurements of MDCK I cells treated with cytochalasin D following establishment of mature barrier formation indicated by plateauing of resistance.

Conclusion

Traditional methods of measuring cellular monolayer permeability lack ease in quantification of barrier function and can be restricted to static measurements. By using a multifrequency approach, ECIS® instruments afford the ability to quantitatively measure the barrier function of cell monolayers continuously in real time without using potentially intrusive labeling techniques such as fluorescence. In addition, during the collection of ECIS® data, the cells remain under incubated conditions. With the ability to measure the cell barrier resistance continually, ECIS® permits researchers to be aware of the barrier maturation in real-time, allowing the implementation of treatments at appropriate timepoints.

Chakraborty, A., de Wit, N. M., van der Flier, W. M., & de Vries, H. E. (2017). The blood brain barrier in Alzheimer's disease. Vascular Pharmacology, 89, 12–18. https://doi.org/10.1016/j.vph.2016.11.008

Guillot, L., Nathan, N., Tabary, O., Thouvenin, G., Le Rouzic, P., Corvol, H., … Clement, A. (2013). Alveolar epithelial cells: Master regulators of lung homeostasis. International Journal of Biochemistry and Cell Biology, 45(11), 2568–2573. https://doi.org/10.1016/j.biocel.2013.08.009

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