Using the ECIS® Model to Isolate Cellular Barrier Function

Electric Cell-substrate Impedance Sensing (ECIS®) has been a well established method to measure cellular behaviors in vitro. When making ECIS® measurements, all of the electrical parameters reported, whether simple impedance, resistance, or capacitance, depend upon the AC frequency used in the measurement. The abstract nature of these electrical variables and their changes with frequency make it difficult to understand how they relate to the actual properties of the cells under study, particularly barrier function. The ECIS® model provides a way to help resolve this uncertainty.

Introduction

Electric Cell-substrate Impedance Sensing (ECIS®) is a cellular impedance method that uses a noninvasive electrical alternating current (AC) sent through gold electrodes at the bottom of ECIS® culture wells. As cells grow over these electrodes, the insulating phospholipid membranes of the cells impede the current, causing a change in voltage that can then be measured (Giaever & Keese, 1984). ECIS® uses multiple AC frequencies that correspond to particular cellular behaviors depending on which frequency (high vs low) is used (see ECIS® Theory application note). Along with a multifrequency approach, ECIS® also uses complex impedance that distinguishes impedance's resistive and capacitive components. By measuring the complex impedance of confluent cell monolayers at several AC frequencies, the ECIS® mathematical model calculates quantities that are morphological attributes of cells in the confluent layer – most notably the cellular barrier function.

Description of the Model: Simplifications and Assumptions

To derive a mathematical model describing a monolayer of cells growing upon an electrode substrate, it is essential to simplify the cell structure and make some limiting assumptions regarding the impedance measurements.

In the model, cells are represented as circular disks hovering a distance, h, over their substrate (Figure 1). Of course, cells in culture vary in shape and are certainly not perfectly circular disks. In addition, anchored cells make some direct contact with their substrate via focal adhesions, but the combined area of these contacts is known to be small relative to the overall cell area.

In addition to this cell morphology generalization, we must assume that the electrode surface is completely covered with the cell monolayer with no open spaces between adjacent cells – a truly confluent monolayer. Finally, the model assumes that the electrode impedance beneath the cell layer has the same impedance at different AC frequencies as that measured from a cell-free electrode.

This simple cell structure and the assumptions make modeling practical and do not unduly affect the final information gleaned from the model.

Description of the Model: Parameters

The development of the differential equations and their solution that mathematically describes the model are not considered in this application note but can be found in the publication where the model was first presented (Giaever & Keese, 1991).

Suffice it to say that in the final solution, it is necessary to know the frequency spectrum of the cell-free electrode. With this information, merely three variables can describe the complex impedance of the cell-covered electrode.

Rb: the specific resistance of the paracellular space between adjacent cells (ohm-cm^2)

Cm: the combined (series) apical and basal membrane capacitance (μF/cm^2)

Alpha (α): a parameter describing the resistance to current flow in the cleft between the basal membrane and the electrode substrate (ohm$^{0.5}$-cm). This quantity increases with the radius of the cell and also with a decrease in the spacing between the cell and its substrate (h)

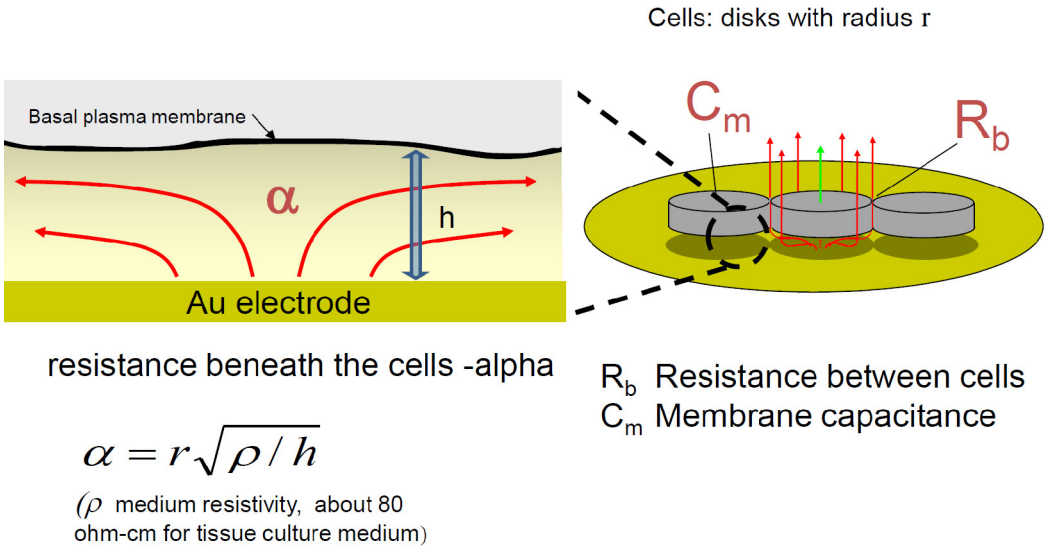

Figure 1: The diagram points out the features of the model. The solid red lines indicate the radial flow of current in the spaces under the cells and through the paracellular space between cells. The green line indicates AC current that capacitively traverses the insulating plasma membrane.

Analyzing data with the ECIS® Model

Important considerations regarding the acquisition of data for modeling:

1. **Impedance must be measured at multiple frequencies.**
2. **At least one cell-free well should be included in data to be modeled.** For the model software to carry out calculations, the complex impedance of a cell-free gold-electrode must be known at all frequencies being used in the measurement.
3. **The model is only valid for cell layers that are confluent** with no open spaces between adjacent cells.

After the initial impedance data have been acquired, the ECIS® software can then model the data provided the previous considerations have been met. The following data are the initial impedance results at an AC frequency of 4,000 Hz for BSC-1 cells treated with 1.25% dimethyl sulfoxide (DMSO) (green trace) versus the cell-free well control (yellow trace). Notice the dip in impedance following the treatment (Figure 2a). To isolate the main cause of the initial impedance decrease caused by the DMSO, the initial data was modeled to separate Rb and alpha (Figure 2b). The modeled results reveal the greater impact on BSC-1 barrier resistance (Rb), reported in ohm-cm^2, as opposed to the attachment resistance (alpha), reported in ohm$^{0.5}$-cm. These results suggest that the DMSO is acting predominantly on the cell-cell junctions of the BSC-1 cells rather than the attachment to the substrate.

Conclusion

ECIS® technology has proven to be a leading in vitro technique for measuring cellular barrier function, amongst many other cell behaviors, and does not require any potentially intrusive labeling techniques. Although most ECIS® resistance data for barrier function can be easily interpretated, some examples have ambiguity of the resistance source. Using a mathematical model, ECIS® software has the ability to distinguish the changes in resistance between the cell-cell junctions and the resistance offered by cell attachment. This allows ECIS modeling to be an extremely effective method to identify compounds that directly affect cell barrier function.

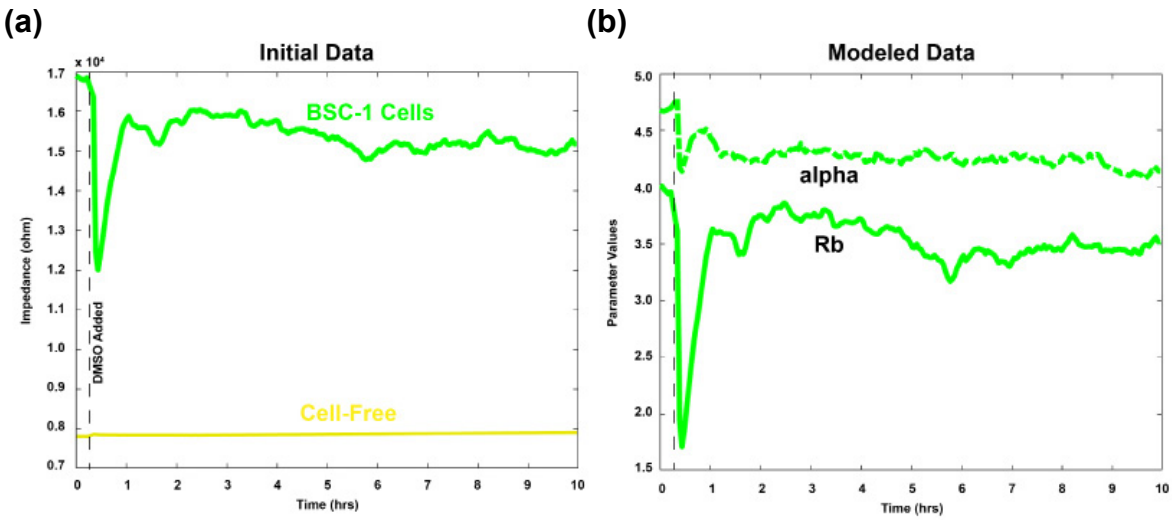

Figure 2: BSC-1 cells treated with 1.25% dimethyl sulfoxide (DMSO). **a)** Impedance values recorded with ECIS® following DMSO treatment. **b)** Impedance data modeled with ECIS® showing parameter values of effected barrier resistance (Rb) as opposed to stable attachment resistance (alpha).

Giaever, I., & Keese, C. R. (1984). Monitoring fibroblast behavior in tissue culture with an applied electric field. Proceedings of the National Academy of Sciences of the United States of America, 81(12), 3761–3764. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=345299&tool=pmcentrez&rendertype=abstract

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. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=52411&tool=pmcentrez&rendertype=abstract