APPLICATIONS

Publications available at www.biophysics.com

ATTACHMENT AND SPREADING
Traditional "counting attached cells assays" can only quantify the number of cells attached to any ECM coating. ECIS® assays give feedback on the strength of the attachment of the cells to the ECM.

CELL PROLIFERATION
As cells proliferate two factors act to change the impedance: cell number and cell morphology. In most instances the cells grow asynchronously and the impedance gradually increases until a maximum when cells become confluent. The impedance change is approximately linear with cell number while the cells are sub-confluent.

DIFFERENTIATION AND STEM CELL BIOLOGY
When cells differentiate they change their behavior allowing ECIS® to follow the events of cell differentiation. While most tools available to characterize stem cells preclude their further use, the label-free non-invasive nature of ECIS® allows for subsequent use of characterized stem cells.

BARRIER FUNCTION
Epithelial cells and endothelial cells regulate the passage of molecules across cell layers. Diseases, especially vascular disease, occur when this function is impaired.

SIGNAL TRANSDUCTION
ECIS® is especially useful to monitor the signal transduction pathways activated by G protein coupled receptors (GPCR). GPCR activation, regardless of the second messenger, results in alterations of the cell's cytoskeletal elements, causing morphological changes.

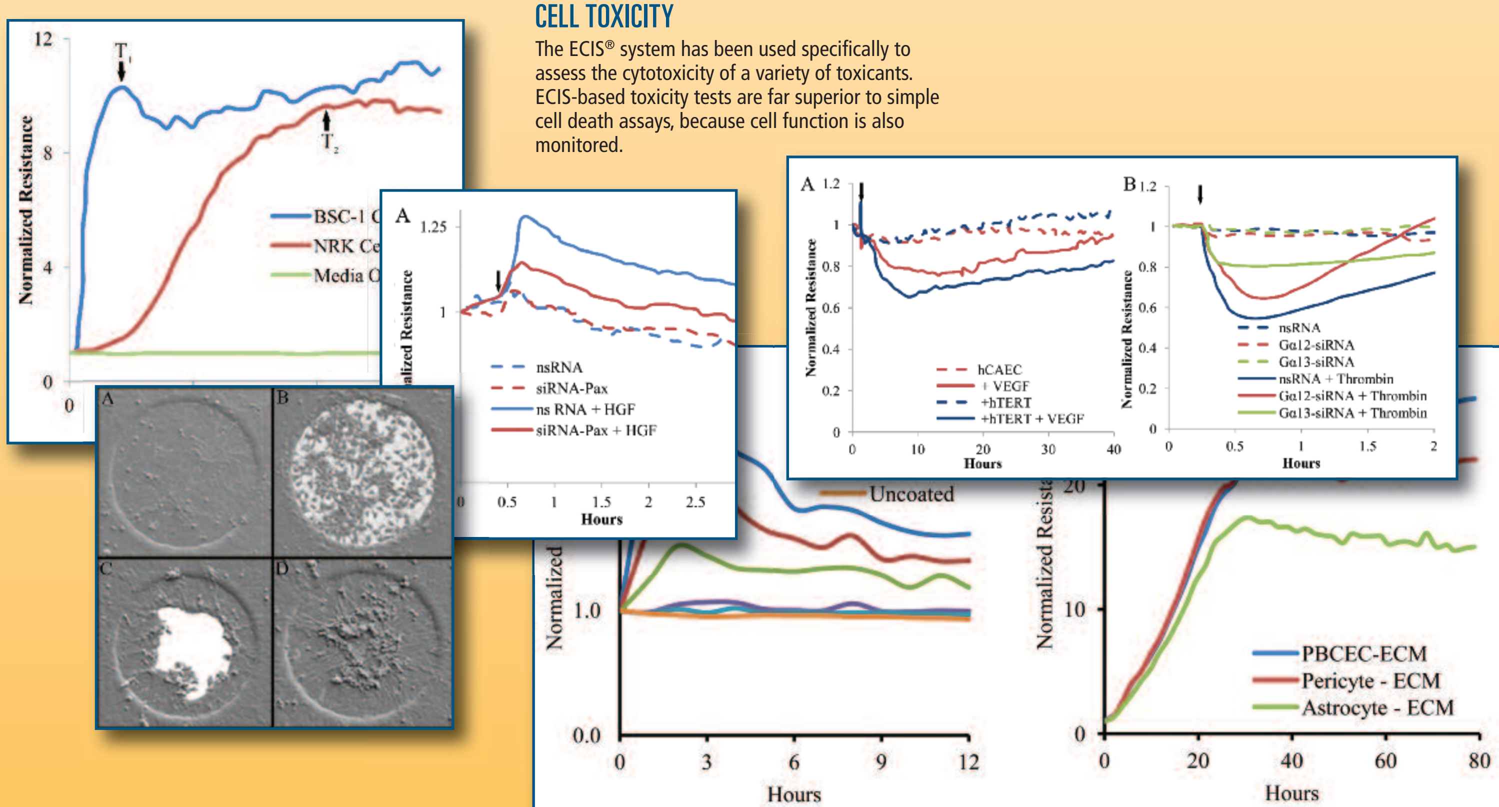
CELL INVASION
ECIS® can distinguish between transmigration mechanisms that leave the monolayer intact from those that disrupt the cell layer. Published examples include metastatic cell and leukocyte trans-endothelial migration, as well as the migration of pathogens such as yeast, anthrax, streptococcus, plasmodium, trypanosomes, and spirochetes.

CELL TOXICITY
The ECIS® system has been used specifically to assess the cytotoxicity of a variety of toxicants. ECIS-based toxicity tests are far superior to simple cell death assays, because cell function is also monitored.

CELL MIGRATION
ECIS instruments include an elevated field mode allowing for electroporation and wounding. The ECIS® wound is precisely defined, as it includes only those cells on the electrode. Additionally, with ECIS® the ECM protein coating is not scraped off and is unaffected by the current.

INFLAMMATION
ECIS recovery-after-wounding assays allow for the discovery of molecules which aid in the process of tissue repair. ECIS barrier function assays specifically measure the response of epithelial and endothelial cells to secreted cytokines and can give indirect information about the binding of immune cells to the epithelium or endothelium.

TEER
Continuous long-term measurement of TEER from under 10 to 10,000 ohm cm2 in up to 24 wells using commercially available 6mm membrane inserts. Fast barrier function dynamics can be accurately monitored.



Applied BioPhysics

Corporate Headquarters:
185 Jordan Road • Troy, NY 12180
1-866-301-ECIS (3247)

www.biophysics.com

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ECIS

ELECTRIC CELL-SUBSTRATE IMPEDANCE SENSING

Impedance Based Cellular Assay

Label Free

Continuous Real Time

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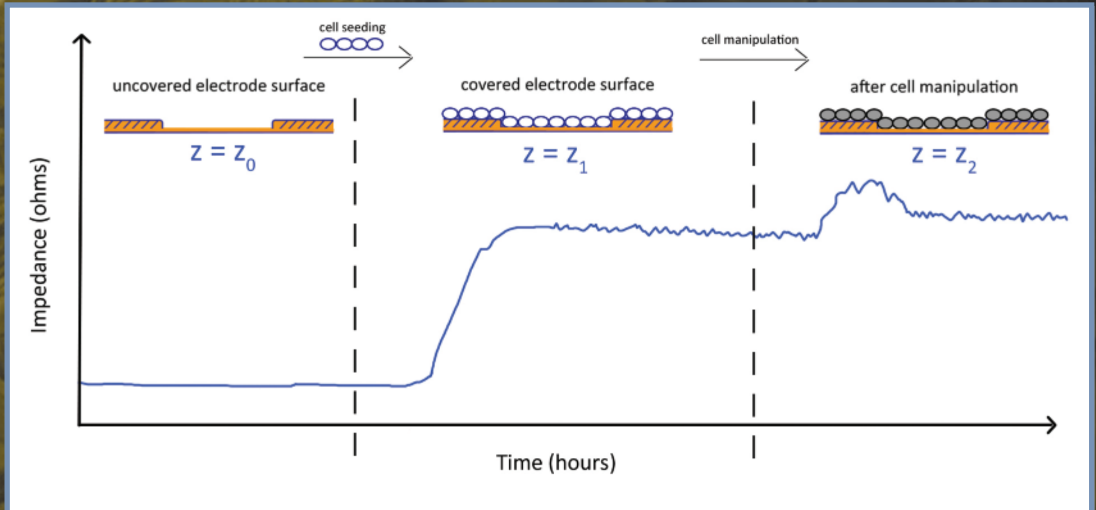
How ECIS works

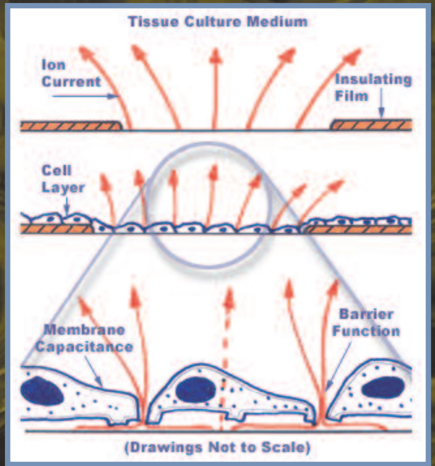


Schematic of an ECIS® experiment starting with a cell free electrode. Once cells are added their presence causes a rise in impedance which plateaus once the cells have reached confluency. At this point the cells can be perturbed and the resulting changes in impedance due to changes in cell behavior are monitored.

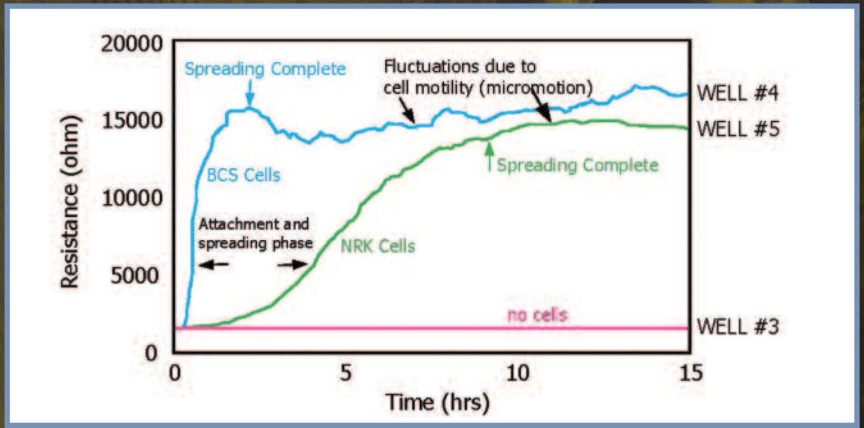
Cell function modulates cell morphology. ECIS® is capable of detecting and quantifying morphology changes in the sub-nanometer to micrometer range. In ECIS® a small alternating current (I) is applied across the electrode pattern at the bottom of the ECIS® arrays (direct current cannot be used). This results in a potential (V) across the electrodes which is measured by the ECIS® instrument.

The impedance (Z) is determined by Ohm's law Z = V/I. When cells are added to the ECIS® Arrays and attach to the electrodes, they act as insulators increasing the impedance. As cells grow and cover the electrodes, the current is impeded in a manner related to the number of cells covering the electrode, the morphology of the cells and the nature of the cell attachment.

When cells are stimulated to change their function, the accompanying changes in cell morphology alter the impedance. The data generated is impedance versus time.



ECIS Data



The instrument can also use a range of AC frequencies from 100-100kHz and complex impedance measurement to determine different cell morphology parameters including barrier function, close contacts, and membrane capacitance.

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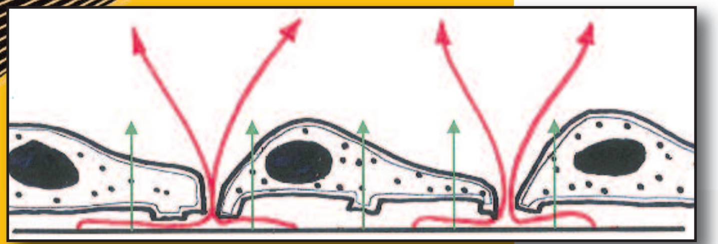
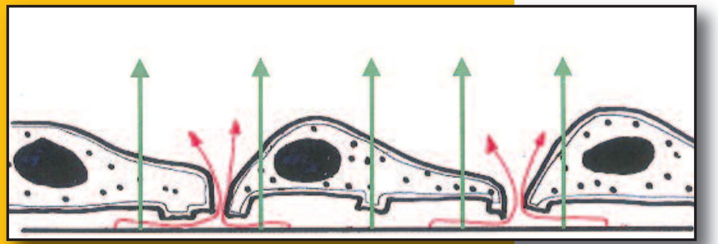
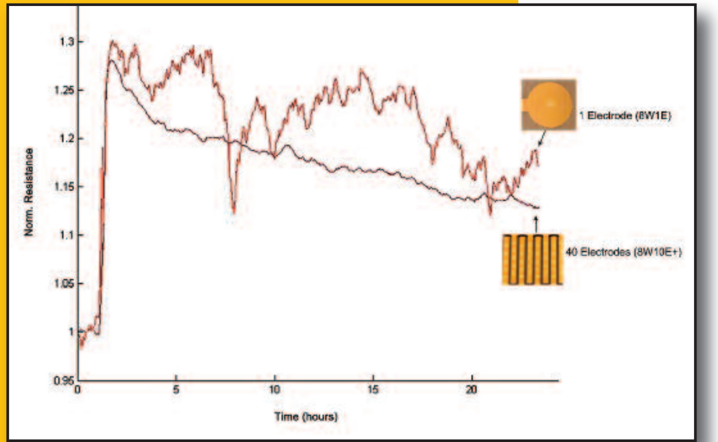


Current flow at low AC frequencies is via paracellular pathways.



Current flow at high AC frequencies is via transcellular pathways.



Response of confluent cell layers to the addition of fresh medium. The plot shows the normalized resistance change as a function of time; the starting values for the 1E plot is 11,500 ohms and for the 10E+ is 1,300 ohms.

How Frequencies Reveal Cell Behavior

To understand why AC frequency is important in ECIS® we have to consider how frequency affects the current paths of cell-covered electrodes. (Note: the total current is maintained constant and voltage changes are measured.) At relatively low frequencies (< 2,000Hz) most of the current flows in the solution channels under and between adjacent cells (red lines).

At higher frequencies (> 40,000 Hz) more current now capacitively couples directly through the insulating cell membranes (green lines).

The high frequency impedance is more affected by cell-coverage, whereas the low frequency responds more strongly to changes in the spaces under and between the cells.

With the more advanced Z instrument, where the impedance is broken down into its components (resistance and capacitance), quantitative information about the cells can be obtained by modeling (Giaever and Keese PNAS 1991).

Using impedance data at multiple AC frequencies the ECIS® model calculates time course changes in:

- The barrier function (permeability) of the cell layer
- The degree of constricted flow of current under the cells
- The cell membrane capacitance

How Electrode Designs Reveal Aspects of Cell Behavior

Small Electrodes

Small electrodes (1E, 10E, 10E+ type arrays) and their layout within the wells ensure that all current passes through the cell monolayer. This allows the ability to analyze data with the ECIS® modeling software to determine barrier function, cell membrane capacitance as well as the spacing between the cell basal membrane and electrode.

Keeping the total surface area of the electrodes small also allows for a relatively low AC current to generate the large electric field necessary to either electroporate or kill the cells in migration experiments.

Small electrodes also provide the ability to monitor the uncorrelated nano-scale morphological changes of individual or small populations of cells (<100), while larger or multiple electrodes provide the averaged morphological response of many cells (1000+).

Large Electrodes

Some experimental protocols, such as cell proliferation, require sparse inoculations leading to a variance of cell density at the bottom of the well. Large electrodes (CP Array) or a large collection of small electrodes (10E+ Array) increases the sampling size resulting in less variability.