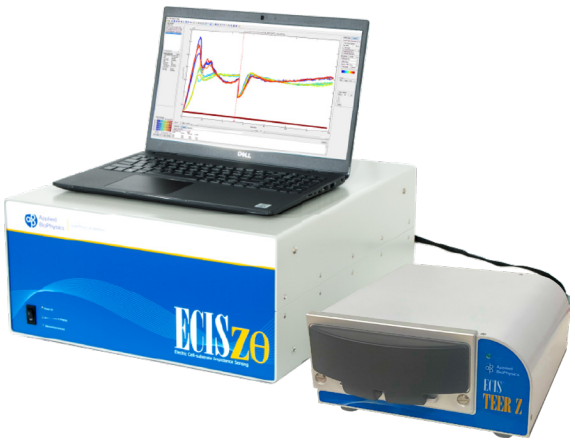
Application Note

Applied BioPhysics
Quantifying Cell Behavior

# Real-time Monitoring of Transepithelial Electrical Resistance (TEER) of Epithelial Cells Following the Inhibition of Actin Polymerization Using the ECIS® TEERZ

Transepithelial electrical resistance (TEER) is an ideal method for quantifying the permeability of epithelial and endothelial cell monolayers and is increasingly being used over typical methods in laboratory settings. Permeability assays have historically required intrusive labeling techniques such as fluorescent dyes and can be time consuming and subjective, whereas TEER measurements require no intrusive labeling and report quantifiable data. As of recent, traditional methods of measuring TEER have been limited to single timepoint measurements and require users to remove the cultured monolayers from tissue culture incubation, subjecting the cells to noncanonical environments. The ECIS® TEERZ allows users to measure TEER continuously in real-time all without removing the cells from the incubator. In this application note, we will display the usefulness of using continuous, incubated TEER measurements while monitoring the barrier dynamics of MDCK I cells upon inhibition of actin polymerization.




## Introduction

Epithelial cells form monolayer sheets that not only act as a defensive barrier but also allow the selective movement of molecules into the interstitial tissue. When monolayers are formed, cells generate adhesions to each other via intercellular junctional protein complexes such as tight junctions, adheren junctions, desmosomes, and gap junctions. The term “barrier function” refers to the paracellular adhesion via the tight junctions that maintain cell polarity and regulate the passage of solutes and water through the paracellular space[1]. These functions have been shown to be largely controlled by the actin cytoskeleton[2].

Transepithelial/endothelial electrical resistance (TEER) is a commonly used method to measure the barrier function of cellular monolayers in vitro and has been widely utilized in biological research. Traditional TEER is conducted by applying a noninvasive alternating current between two electrodes resulting in current flowing between the paracellular space of cell monolayers as well as through the space between the substrate and the basal membrane.

Applying Ohm’s law (V = IZ), the impedance of the current traveling though the monolayers is calculated from the ratio between the voltage and current. TEER is then normalized by multiplying this impedance by the measured substrate area. The cell layer permeability is inversely related to these TEER levels. Among the many advantages of measuring TEER in cell monolayers, one of the most important is that labeling techniques such as dyes including fluorescence, that may be intrusive to the cell’s natural functioning, are not necessary.

Until recently, using TEER to quantify monolayer permeability has been constrained by the lack of tissue culture conditions such as temperature and pH due to the requirement of removing the cells from the incubator to apply the measurement. Additionally, traditional TEER measurements only measure a single point in time as opposed to a continuous measurement, making data collection cumbersome and ambiguous.

Conventionally, TEER is calculated from total impedance (Z), often referred to as simple impedance. Total impedance may be a contribution of multiple pathways of alternating currents in a circuit, namely resistance (R) and capacitive reactance (Xc), both measured in ohms. Cell monolayers can be modeled as a parallel resistance-capacitance (RC) circuit, and each pathway of the current's contribution to the total impedance is largely determined by the frequency (Hz) of the alternating current: lower frequencies cause the majority of the current to flow in what we term as the resistive pathway that traverses through the paracellular space, and higher frequencies cause the majority of current to flow the capacitive pathway through the cell membranes (Figure 1). Since TEER is intended to measure the resistance of the cell-cell junctions, utilizing the resistive pathway through low-frequency, alternating current is

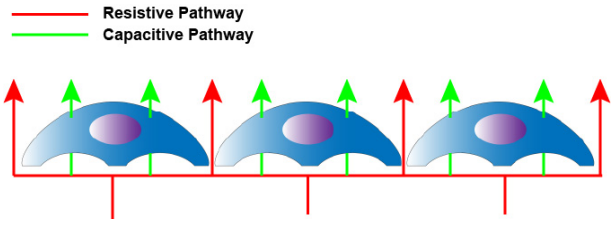



**Figure 1:** Schematic model representing alternating current pathways through cellular monolayers. The resistive pathway (red) travels around and through the paracellular space whereas the capacitive pathway (green) capacitively couples through the cell.

ECIS® technology has the ability to isolate these components of impedance into separate pathways for more detailed analysis of the cell behaviors being measured[3]. For more information on ECIS® technology, see our application note Electric Cell-substrate Impedance Sensing: A Label-free Method to Continuously Monitor Cell Behaviors In Vitro.

While standard ECIS® was developed for solid-substrate culture plates, the ECIS® TEERZ utilizes ECIS® technology to specifically measure the TEER of cells that are seeded on permeable filter inserts by measuring both the series resistive and capacitive pathways. Following this, ECIS® software then mathematically converts these to parallel resistance and capacitance to better mimic the two pathways we have described. This allows the measurements to account for the loss of current flow to the capacitive pathway when cell barriers reach a very high level of resistance[4]. The following figure follows the progression of resistance, capacitive reactance, and TEER values of MDCK1 cells (Figure 2). Notice the delay in increase of the capacitive reactance following a ~1,000 ohm increase in resistance (Figure 2a,b). By compensating for the loss of current to the capacitive pathway in the parallel circuit, the true resistance caused by the cells is used to calculate TEER values (Figure 2c).

Similar to the way chopstick-style impedance probes are inserted into the filter inserts and the basal wells, the ECIS® TEERZ uses two medical-grade stainless steel pin electrodes that flank the permeable insert in the same manner. By using the electrolytes in the cell medium as a conduit, the alternating current then travels between the two electrodes, hence through the permeable filter membrane and cell layer.

In this application note we will demonstrate the usefulness of measuring TEER continuously with the ECIS® TEERZ while monitoring the effects of inhibiting actin polymerization in epithelial cells.

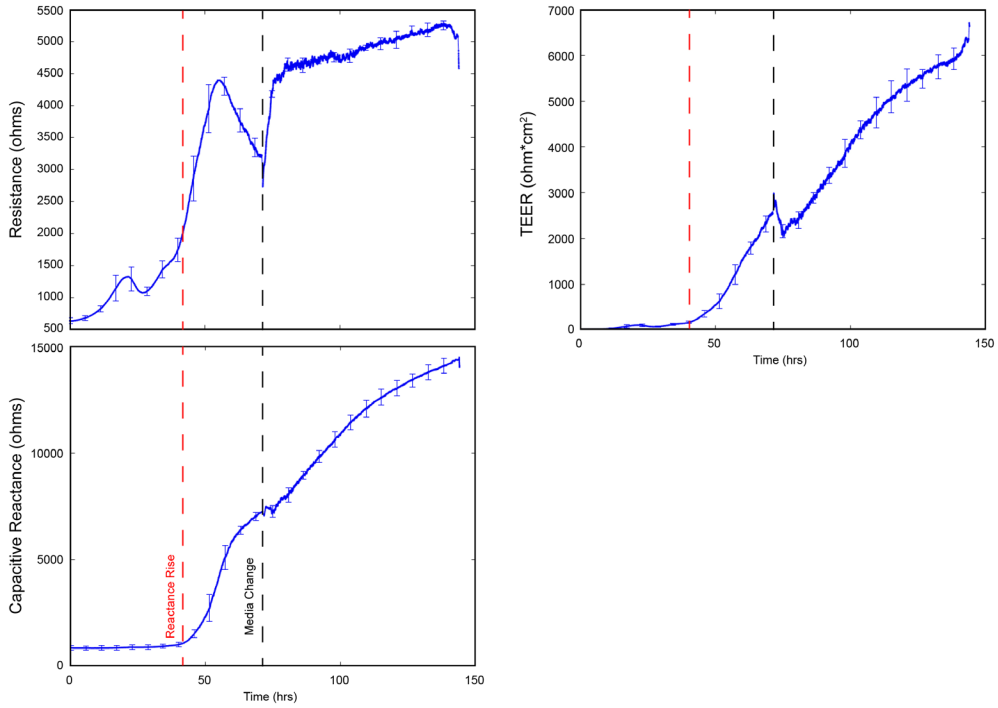



**Figure 2:** Loss of current to the capacitive pathway when cell barrier resistance increases roughly 1000 ohms. a) MDCK1 cells show as a change of resistance increases to ~1000 ohms, b) capacitive reactance begins to increase, indicated by the red dashed line. c) When calculating TEER, resistance is paralleled thereby compensating for the loss of current to the capacitive pathway.

## Methods

*Cells and Reagents*

Madin-Darby canine kidney cells (MDCK1) (ATCC) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Sima-Aldrich) containing 10% Fetal Bovine Serum (Sigma-Aldrich) and 50 µg/ml of gentamicin (Sigma-Aldrich) at 37° C and 5% $CO_2$. Cytochalasin D (Sima-Aldrich) is an actin polymerization inhibitor[5] and was diluted with dimethyl sulfoxide (DMSO) (Sigma-Aldrich) to 1 mg/mL and used at final concentrations of 0.13, 0.25, and 0.5 µg/mL (n = 4).

*Cell Seeding*

MDCK1 cells were seeded on a Corning HTS Transwell 24-well Permeable Support (Corning) plate at a density of 1.5 x $10^5$/cm$^2$ at 200 µL in the upper chamber and media only at 1,000 µL in the lower chamber.

*Transepithelial Electrical Resistance*

Transepithelial electrical resistance (TEER) was measured using the ECIS® Z-theta with ECIS® TEERZ Array Station and TEERZ 24-well Cassette (Applied BioPhysics). The TEERZ uses Electric Cell-substrate Impedance Sensing (ECIS) technology and collects complex impedance measurements on cell monolayers seeded on transwell permeable inserts with two stainless steel dipping pin electrodes. Complex impedance is converted into TEER (ohms*cm$^2$) via ECIS® Software as previously described. The TEERZ allows continuous and label-free measurements of TEER while the cell samples remain in the incubator.

## Results

TEER was monitored continuously under incubated conditions for a period of 160 hours. MDCK1 cells were seeded at 50,000 cells per well and were monitored for ~72 hours before

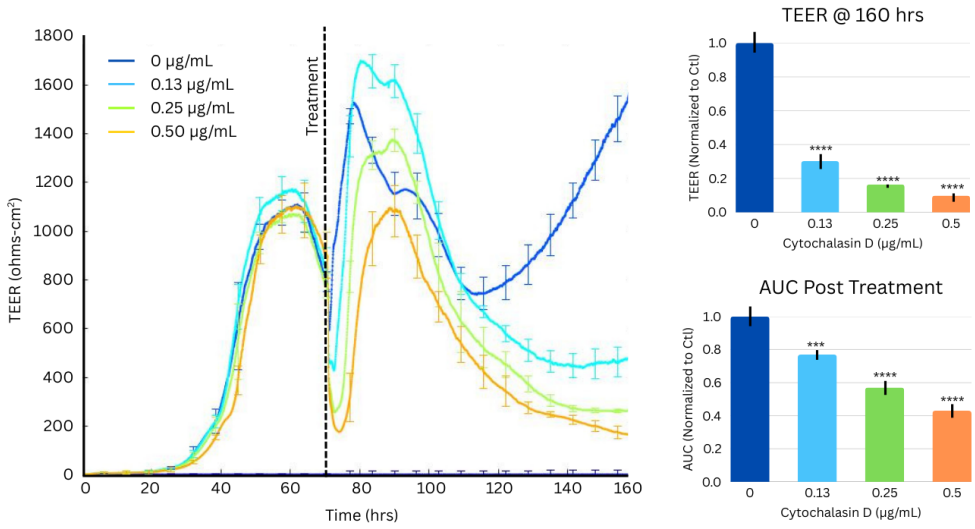



**Figure 3:** Continuous TEER effects after inhibiting actin polymerization in epithelial cells. a) Real-time TEER measurements of MDCK I cells treated with Cytochalasin D. The black dashed line represents time of treatment addition. Mean +/- SD, n = 4 replicates. b) Bar graph displaying TEER at ~160 hrs. Mean +/- SD, unpaired t-test. b) Bar graph displaying area under the curve of each treatment group following treatment addition. Mean +/- SD, unpaired t-test.

adding the actin polymerization inhibitor cytochalasin D to the upper chamber. Following the treatment, the TEER values showed a decrease proportional to the increasing concentrations of treatment given showing the biggest decrease with 0.5 µg/mL. Interestingly, following the initial decrease, TEER values in the treated wells mimicked the same behavioral pattern as the control by increasing to similar TEER values as the control while maintaining the dose-dependent, relative response until finally diverging from the control well's behavior by again decreasing in TEER values with dose-dependency (Figure 3a,b). Since the data is in real-time, it is useful to compare the samples by calculating the area under each curve (AUC) given the dynamic changes post-treatment. The AUC data reveals a linear-like relationship corresponding with treatment dosage (Figure 3c).

## Conclusion

Transepithelial electrical resistance (TEER) has proven to be one of the most useful methods to measure the barrier function of cell monolayers in vitro given its ability to monitor the cell layer permeability without the need for potentially intrusive labeling techniques. Until recently, TEER has mostly been limited to single timepoint measurements being taken outside of incubated conditions therefore placing the cells in a less canonical environment. The new ECIS® TEERZ holds the capability of measuring TEER continuously in real-time, while the cells remain inside of the incubator. By using complex impedance, the ECIS® TEERZ isolates the resistive pathway of total impedance, revealing the true resistance caused by the cell monolayers for TEER calculations.

When inhibiting actin polymerization, epithelial cell barriers show an increase in permeability in a concentration dependent manner as has previously been shown. Since the ECIS® TEERZ can monitor TEER values continuously in real-time, area under the curve calculations can be applied, highlighting the unpredictable permeability dynamics, and opening up new targeted avenues of research and discovery.

## References

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