Automated Wound-Healing Cell Migration Assays with ECIS®

Wound-healing migration assays in tissue culture are typically conducted with mechanical measures to remove cells in specified areas, and as cells migrate to repopulate this area, a migration rate can be calculated. This process can be difficult to reproduce and measurements tend to be inconsistent. Electric Cell-substrate Impedance Sensing (ECIS®) is a real-time, impedance-based method to study multiple behaviors of cells when grown in tissue culture without the use of intrusive labeling techniques. In this application note, we will discuss using ECIS® to electrically wound cell monolayers and subsequently measure the migration of the cell layer into the wound while the cells remain incubated.

Introduction

Wound-healing assays have typically been employed to measure the coordinated migration of cell monolayers. To accomplish this in vitro, cell-free areas are created in confluent monolayers, commonly by mechanical means, where a pointed object is used to scrape a narrow open area (wound) in the monolayer. Following the creation of a cell-free area, the surrounding cells migrate into the wound to reestablish confluency, and their progress is followed with microscopy. Unfortunately, these types of assays are difficult to repeat consistently, and quantifying the results is often ambiguous. Applied BioPhysics Inc. has developed a method to carry out this measurement using an electrical approach based on Electric Cell-substrate Impedance Sensing (ECIS®) 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 at the bottom of ECIS® tissue culture wells. As cells grow over these electrodes, the current is impeded by the insulating cell membranes, and the resulting impedance data are reported in graphical format (Figure 1).

Automated Wound Healing & Cell Migration with ECIS®

The ECIS® Wound Healing Assay replaces the traditional "scratch" or "scrape" assay. Instead of disrupting the cell layer mechanically with a toothpick, needle or pipette tip and following the cell migration into the wound with a microscope, ECIS® employs electric signals to both wound and monitor the healing process. Also, the assay is completely automated, requiring a minimal amount of labor. Both cell wounding and measurements of the subsequent healing process are carried out under computer control while the cells remain incubated.

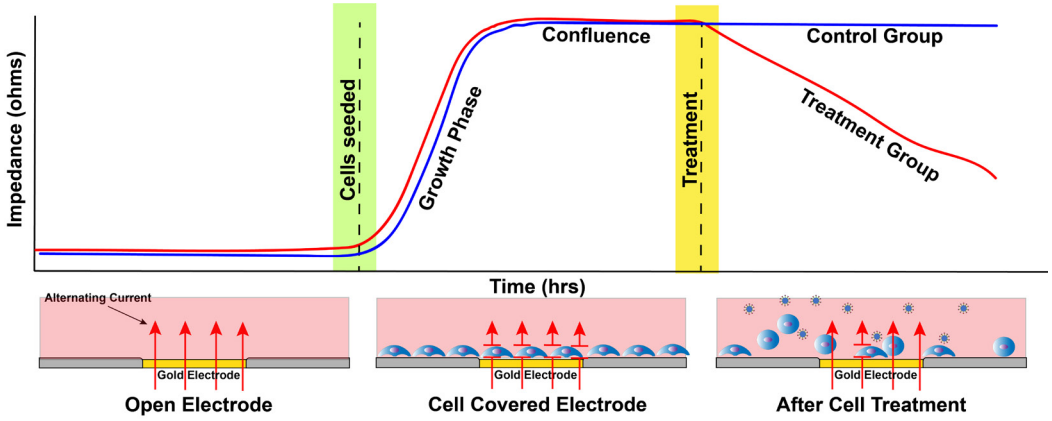

Figure 1: Schematic representation of ECIS® data with impedance vs time. As cells grow and cover electrodes, impedance data rises proportional to cell coverage of the gold electrodes.

To accomplish the wounding, the ECIS® instrument increases the non-invasive measuring electrical current ~1,000 fold, resulting in cell necrosis. The theory explaining this phenomenon is electroporation, and for the wounding application we are generating long term and irreversible electroporation. To induce these pores and cell death, a high current must be sustained across the cell layer for a sufficient time (normally a few seconds). The exact current or time duration vary with cell type, so optimal wounding conditions are generally determined for each specific cell type.

ECIS® electrical wounding is only directed at the small population of cells in contact with the active 250-micrometer diameter gold electrode, producing a well- defined 250-micrometer wound (Figure 2a) that can be verified both with the ECIS® measurement and with vital staining (Keese, Wegener, Walker, & Giaever, 2004). Once the ECIS® instrumentation electrically wounds the cells, it returns to normal measurement mode to immediately follow the healthy neighboring cells as they migrate inward to replace their dead cohorts (Figure 2b).

In addition to the precise definition of the wound, another advantage with electrical wounding is that the adsorbed protein coating of the electrodes is unaffected by the high current (Hung et al., 2022). With traditional scratch assays, the mechanical stress to the cell layer not only damages the cells, as intended, but also tends to damage the underlying extracellular matrix (Kroening & Goppelt-Struebe, 2010), causing migrating cells to spread over an undefined surface.

Multifrequency Approach to Cell Migration

The ECIS® Cell Migration Assay follows impedance after lethal electroporation and has three ECIS® phases: lag, fast recovery, and slow recovery. We correlate these phases with a cell transition, cell migration, and reestablishment (annealing) of cell-cell interactions, respectively. Of the three phases of recovery, only the transition and migration phases would be observable by microscopy-based methods. The annealing phase is uniquely observable with ECIS® and best resolved by monitoring resistance at low frequencies (<4000 Hz). The transition and migration phase are best measured by capacitance or impedance at high frequency (>32,000 Hz). At high frequencies, the measurement is less sensitive to cell-cell interactions, resulting in impedance or capacitance curves dominated by cell migration and simple coverage of the electrode (Figure 3).

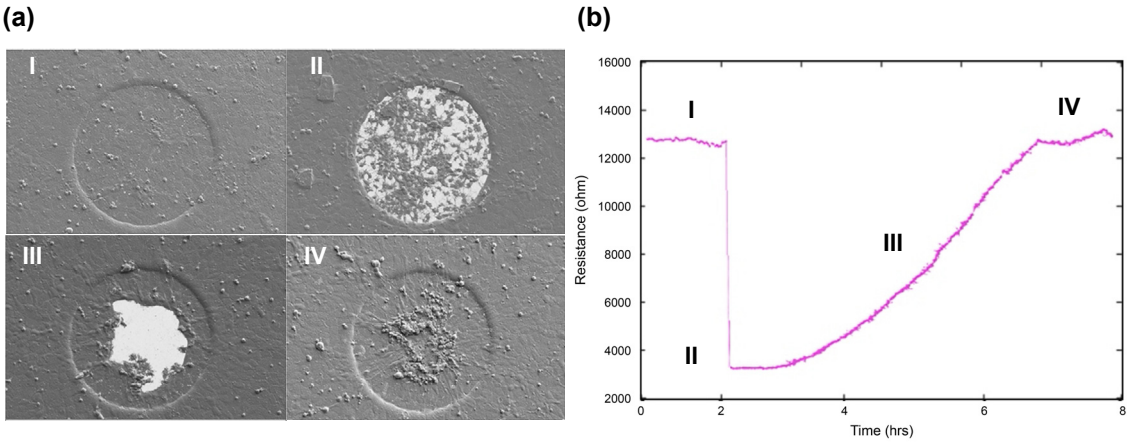

Figure 2: ECIS electrical wounding and subsequent migration. **(a)** Scanning electron microscope images of an ECIS® array electrode of NRK cells I) prior to wounding, II) immediately after wounding, III) 4 hours after wounding, and IV) 8 hours after wounding. Images courtesy of Dr. Joachim Wegener and Dr. Vanessa Heitmann. **(b)** ECIS® graphical output of electrical wounding with associated stages of migration to SEM images.

The proper units for cell migration experiments are distance per unit time. ECIS® Cell Migration Assays directly measure the recovery of impedance in a very precise cell free area. Full recovery of the impedance consists of both the migration phase and the annealing phase. To convert to distance per unit time requires that the migration phase be differentiated from the annealing phase. In general, this is accomplished by analyzing the recovery by capacitance or impedance at high frequencies where the annealing phase is a minor contributor in the recovery. In the example in Figure 3, the high frequency capacitance required approximately 10 hours for the cells to migrate to the center of the circular electrode (radius 125 micrometers) hence a calculated migration rate of 12.5 micrometers/hour.

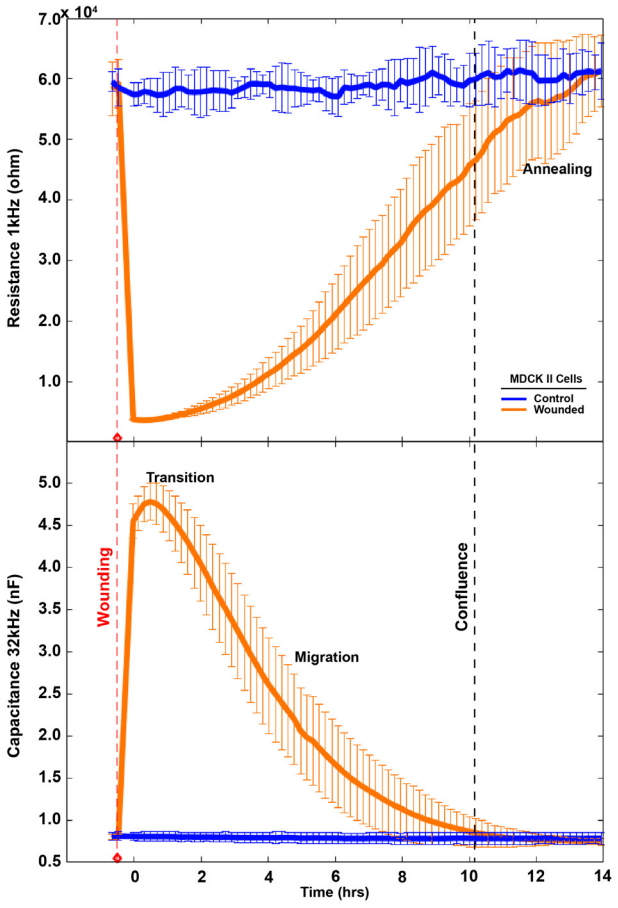

Figure 3: ECIS® cell migration curves shown as change in resistance and capacitance in the same samples. Resistance is measured at 1 kHz AC frequency following the electrical wounding of the cell monolayer representing attachment and barrier resistance whereas a decrease in capacitance at 32 kHz represents cell coverage as the cells migrate onto the cell-free electrode .

The Electric Fence Method

Although the ECIS® electrical wounding method is effective for most cell types, some cells can be more difficult to wound than others. An alternative ECIS®-based method for migration assays is the Electric Fence method. Unlike the wounding assay described above, the Electric Fence is not used with established confluent monolayers, in addition, rather than applying a single current pulse, the Electric Fence continues sending high current pulses, usually one short burst every five minutes, until the feature is switched off. For cells that are difficult to electrically wound, this feature can be turned on at the start of the experiment, allowing for freshly seeded cells to grow and form confluence around the electrode but avoiding attachment directly upon the electrode due to the ongoing elevated current pulses. Once the cells have achieved confluence outside of the electrode, the Electric Fence can be turned off, and the bordering cells now are able to migrate over the electrode while the normal weak measuring current remains on to follow their progress. The resulting impedance changes are then displayed in graphical format (figure 4).

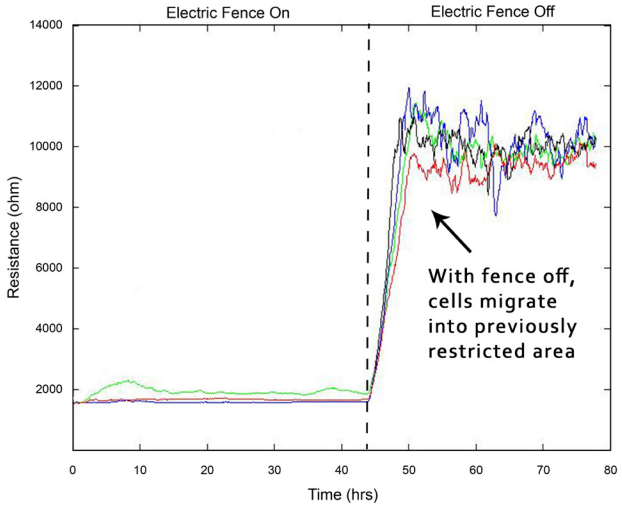

Figure 4: MDCK II cell migration using the ECIS® Electric Fence. The fence engaged at the beginning of the experiment and disengaged at ~43 hours, allowing cells to now migrate over open electrode causing resistance levels to rise until plateauing representing confluence.

Conclusion

Wound-healing assays, via the traditional scratch assay, pose problems of repeatability, difficulty in quantification, and damage to the underlying extracellular matrix. With the ECIS® Wound-Healing Migration assay, cells are electrically wounded with an elevated electrical current, causing lethal poration of the cell membranes. The electrical wounds are perpetrated on precisely sized areas (electrodes) and can be repeated with consistency, ensuring the researcher is measuring the same area with each subsequent wound. ECIS® instruments also utilize a multifrequency approach using resistance and capacitance to analyze the phases of transition, migration, and annealing (cell-cell junctions) during the wound-healing process. If a cell type fails to behave as described in the standard assay, ECIS® offers the alternative Electric Fence where cells are made to avoid the electrode while growing to full confluency on the neighboring substrate. When confluency has been reached in this region, the researcher turns off the Electric Fence and quantifies the migration of cells upon the previously fenced electrode. ECIS® instruments provide researchers the opportunity to consistently monitor cell migration in vitro, continuously, in real-time, and without relying on poorly defined and arduous techniques such as scratch assays.

Hung, Y. H., Chiu, W. C., Fuh, S. R., Lai, Y. T., Tung, T. H., Huang, C. C., & Lo, C. M. (2022). ECIS Based Electric Fence Method for Measurement of Human Keratinocyte Migration on Different Substrates. Biosensors, 12(5), 1–15. https://doi.org/10.3390/bios12050293

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