ECIS

Electric Cell-substrate Impedance Sensing

Applied BioPhysics

Cell Migration Assay

Theory: The ECIS instruments use mili-Ampere currents in order to generate 1- 10 volt potentials across the cell monolayer. This potential across the cell monolayer is sufficient to induce pores in the cytoplasmic membrane. If this pulse is applied in a brief pulse (<0.5 sec), then the effect is to reversibly electroporate the cells. If the pulse is great enough (> 1V across the cell membrane) or sustained (~> 5 seconds) then the cells die by irreversible electroporation. This results in a very defined area of dead cells, and a resulting drop in impedance. Then cells along the periphery of the electrode then migrate to repopulate the electrode, healing the electrically induced wound. This recovery after electrical wounding is the ECIS Cell Migration Assay.

The target voltage for irreversible electroporation is greater than 1V across the cell membrane. ECIS instruments allow for direct control of the current. To calculate the voltage generated across the cells (Vcells) simply multiply the current (I) by the impedance due to the cells at the wounding frequency (Zf).

$$V_{cells} = I \times Z_f$$

Thus if a current of 1mAmp is selected for wounding and the impedance in the wells at 48 kHz is 7,000 Ohms then we would generate a 5 V potential across the cells (Cell free Impedance is ~2000 Ohms, thus 5000 Ohms are due to the cells). There are two ways to get to the target voltage. Increase I or increase Zf. Zf increases inversely with frequency so higher Z may be achieved by going to lower frequencies. However the chance of electrode damage increases at lower frequencies, as thermal effects become significant. A floor of 20 kHz is recommended for wounding experiments.

Array selection: Any ECIS array is capable of conducting the wounding current, but the different geometries will generate different effective potentials across the cells. We recommend the 8W1E or the 8W2LE arrays for ECIS Cell Migration Assays as these array types are capable of generating the highest potentials across the cells. However some researchers have preferred the 8W10E arrays, in which the resulting data is the combined response of 10 wounding and migration sites. The 8W2OE array provides for a longer migration distance, at two oblong wounding/migration sites, and generates potentials across the cells approximately that of the 8W10E arrays. Table 1 summarizes the types of electrodes that we offer for wounding experiments.

Catalog Number	Description	Migration Distance
8W1E	8 Wells w/ 1 circular electrode each	125 um
8W10E	8 Wells w/ 10 circular electrodes each	125 um
8W2LE	8 Wells w/ 2 linear electrodes each	75 um
8W2OE	8 Wells w/ 2 oblong electrodes each	250 um

Table 1

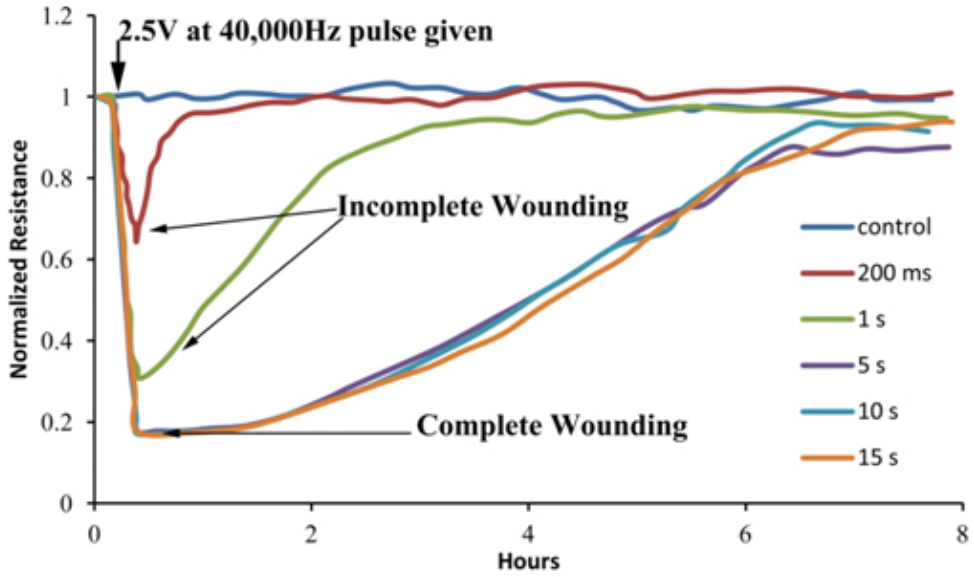

Figure 1A- 2.5 V pulse was given to BSC-1 cells growing on 8W1E electrodes for the indicated time. Curves are normalized to the Resistance just prior to wounding.

Optimizing Wounding Parameters: The ECIS Cell Migration Assay is accomplished by creating a cell free area on the electrode by electrically killing the cells on the electrode. This is accomplished by generating a large potential across the cells, generating pores in the cell membrane. The theory explaining this phenomenon is electroporation and for the wounding application we are generating irreversible electroporation. An introduction to this technology is given in a review by Boris Rubinsky 19.

Briefly, the cells are killed by pores forming in the cell membrane that exist long enough to induce lysis of the cell. To induce these pores a sufficient potential for a critical time must be sustained across the cells. However the exact potentials or time durations vary with cell types, so optimal conditions need to be found for each cell type. In addition, in some instances over electroporation can cause fusion of the cells or damage the electrode. Thus we recommend that the minimum conditions to ensure complete wounding be used for ECIS Cell Migration Assays.

To establish the minimum conditions for complete cell wounding by irreversible electroporation, an initial experiment like that of figure 1 should be carried out. In this experiment a current is selected to deliver 2.5 V at 40,000 Hz to an 8W1E array for 0.2, 1, 5, 10, and 15 seconds in different wells. The trace corresponding to 200ms and 1s illustrates incomplete wounding. Complete wounding occurs when the 2.5 V pulse is applied for 5 seconds or longer. Subsequent ECIS Cell Migration Assays for these cells should therefore have 2.5 V at 40,000 Hz for 5 Seconds as their wounding parameters.

Note: For some cell types that are resistant to electrical wounding, repeated 10 second pulses have proven to be more effective than a single 30 second or longer pulse. If you are finding that you need longer high voltage pulses in order to wound your cells, we recommend trying repeated pulses. In some instances it may be necessary to apply the high voltage pulse in a low osmolarity lysis medium.

Voltage Calculation: To calculate the voltage generated in a well, read the impedance of that well at 40,000 Hz. For an 8W1E electrode it will be between 1000 and 10,000 Ohms depending on the cell type. Multiply this number by the wounding current (500uA - 3000uA) to get the Voltage.

The following table is a short list of values from the ECIS literature that have given satisfactory wounding results for various cell types.

Cell	Array	Volts	Freq.	Seconds	Distance
16HBE14o	8W10E	5.00	40,000	30	6
A549	8W10E	5.00	40,000	30	6
ARPE19	8W1E	4.00	40,000	10	3
BEAS-2B	8W10E	5.00	40,000	30	6
BSC-1	8W1E	2.50	40,000	5	10
CACO2-BBE	8W1E	4.50	40,000	30	2
CAEC	8W1E	~10	60,000	20	15
Calu-3	8W1E	6.00	30,000	60	12
DU-145	8W10E	6.00	?	30	18
HaCaT	8W1E	5 - 12	40,000	0.2	7
HaCaT	8W10E	6.00	?	30	9
HeLa	8W10E	4.00	45,000	?	4
HeLa	8W10E	4.00	45,000	10	5
HepG2	8W1E	3.50	40,000	30	11
HPAEC	8W1E	3.00	40,000	10	1
Huh7	8W1E	3.50	40,000	30	11
HUVEC	8W1E	5.00	40,000	30	13
HUVEC	8W1E	6.00	60,000	60	13
HUVEC	8W1E	3.00	40,000	15	16
MDA-MB231	8W10E	6.00	?	30	8
MDCK	8W1E	5 - 12	40,000	0.2	7
NCI-H292	8W10E	5.00	40,000	30	6
NHBE	8W1E	6.00	30,000	60	12
NRK	8W1E	2.50	40,000	10	10
PBEC	8W10E	5.00	40,000	30	6
PC-3	8W10E	6.00	?	30	18
RPE	8W1E	2.50	40,000	10	14
UNC-CF1T	8W1E	6.00	30,000	60	12

Data Analysis: The ECIS Cell Migration Assay is a recovery after irreversible electroporation experiment and has three ECIS phases; lag, fast recovery, and slow recovery. We currently correlate these phases with a cell transition, cell migration, and re-establishment (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 only 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 (>40,000 Hz). At high frequencies the measurement is less sensitive to cell-cell interactions resulting in impedance or capacitance curves dominated by cell migration.

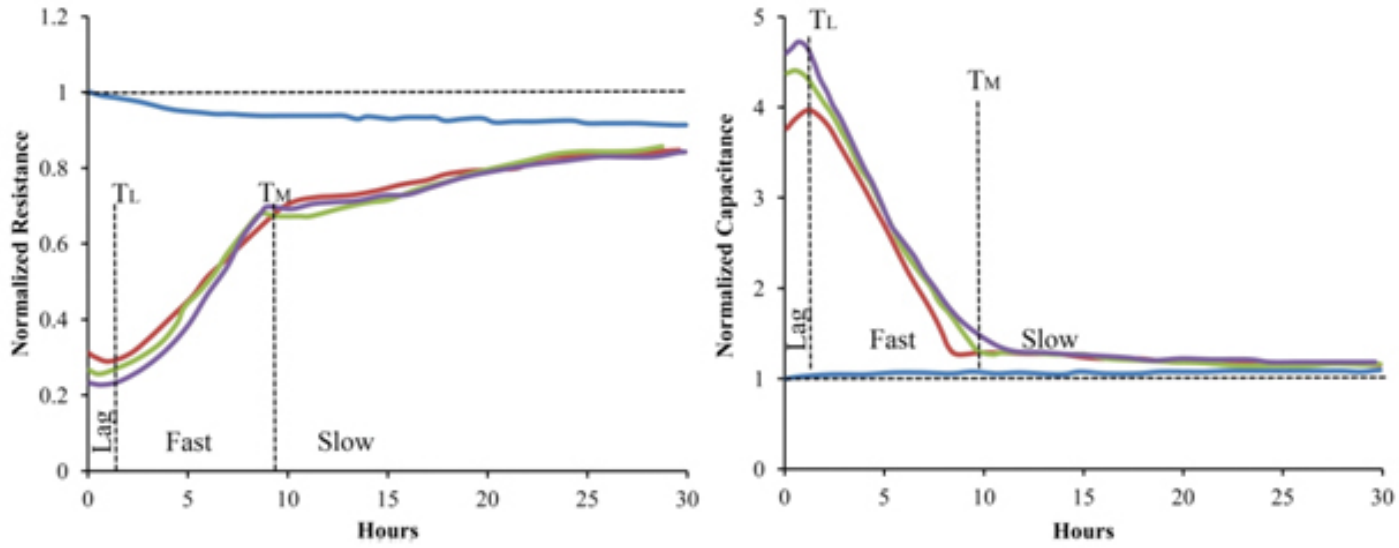

Figure 2- ECIS Cell Migration Assay showing recovery as a function of normalized resistance and normalized capacitance. Data is normalized to the control well starting value. The three phases of recovery after irreversible electroporation are demarcated in both instances. Confluent BSC-1 cells were wounded by 2.5 V for 5 sec at 40,000 hz just prior to the start of ECIS recordings.

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.

Once the migration phase has been identified the conversion to distance per time is made by dividing the migration distance of the array type (Table 1) by the time between the wound and end of the migration phase(T_M). This number will most closely compare with optical measurements in typical scratch assays. However, as this number incorporates the transition phase (T_L), it underestimates the actual migration rate of the cells. A truer estimate of a cells migration rate would use the time interval from the beginning of the migration phase to the end ($T_M - T_L$).

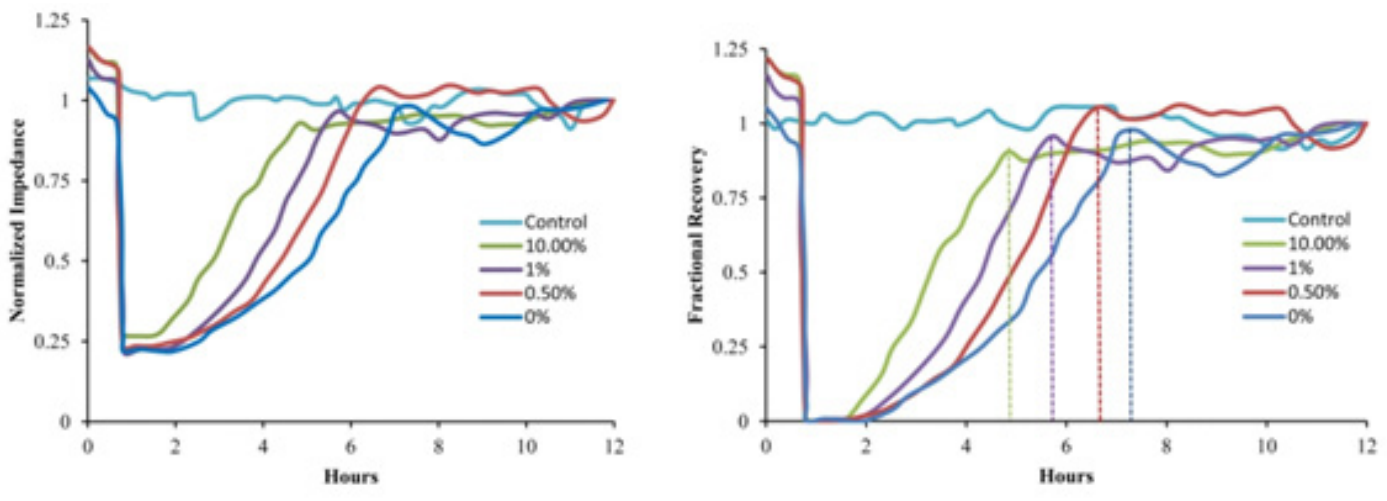

Figure 3- ECIS Cell Migration curves shown as change in normalixed impedance as a function of time (A) or fractional recovery as a function of time (B). Data is dervived from (10) of BSC-1 cells recovering after a 2.5 V 15 second pulse at 40,000 Hz. Cells were cultured in normal media supplemented by the indicated amount of serum.

If only ECIS based data will be compared, then just the time interval between wounding and a common end point is sufficient for comparing data sets across experiments. As long as the electrode size is kept constant the migration distance is always the same. Thus TM becomes a sufficient metric by which to characterize the cell migration.

For displaying the results of migration data it is generally preferable to show the richness of the time course data.

Typically people report their cell migration experiments in graphical format like that of figure 3. Here wells recover from the wounding pulse in a dose dependent manner on the percentage of serum in the media. While showing the results as normalized impedance, resistance, or capacitance is acceptable, it is generally more informative to express results as a fractional recovery. The advantage of this transformation is that recovery measured by capacitance (figure 2b), which occurs by going from large values to low, can be inverted and then has a display similar to impedance or resistance which go from low values to high. The following table shows the conversion formulae for converting impedance, resistance, and capacitance to fractional recovery units.

Impedance Value	Formula to Convert to Fractional Recovery
Impedance	Z(t)-Z(min)/[Z(0)-Z(min)]
Resistance	R(t)-R(min)/[R(0)-R(min)]
Capacitance	C(max)-C(t)/[C(max)-C(0)]

Z(t), R(t), and C(t) are the impedance, resistance or capacitance value at time = t. Z(min) and R(min) are the minimal impedance and resistance values immediately after wounding. C(max) is the maximal capacitance value directly after wounding. Z(0), R(0), and C(0) are the initial impedance, resistance, or capacitance values immediately prior to wounding.

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