Array Stabilization

ECIS® arrays are oxygen plasma etched the day they are shipped from Applied BioPhysics. This step sterilizes the arrays and also cleans the gold resulting in a clean, pristine electrode. Over time, small molecules in the atmosphere adsorb to the gold surfaces resulting in lower capacitance of the electrodes. This lowered capacitance affects both the impedance and resistance measured across the electrodes. When exposed to tissue culture medium, these molecules gradually desorb and the impedance values eventually return to those associated with clean electrodes.

In addition to the desorption of these inorganic small molecules, proteins in the medium bind to the gold electrode and to the plastic surfaces of the wells. This creates a new equilibrium capacitance of the electrodes. To assure these processes take place in a timely manner, we strongly recommend that researchers take measures to stabilize the gold before cell inoculation. There are two approaches to accomplish this – cysteine treatment and electrical stabilization.

Θ Note: Cysteine treatment may interfere with coating of the arrays with ECM proteins. Therefore if ECM coatings are to be used, they should be applied prior to the array stabilization.

Cysteine treatment

Our preferred way to stabilize the gold electrode is with the amino acid cysteine. The cysteine will form a covalent sulfur-gold linkage with the electrode surface, likely displacing any unwanted small molecules that have adsorbed to gold surface over time, stabilizing the impedance. This cysteine layer provides a hydrophilic substrate that will allow cell attachment and spreading.

This treatment enhances experimental repeatability with minimum variation between ECIS® wells. The protocol for cysteine coating of an array is as follows:

1. Flood the wells with 200 µl of 10 mM sterile solution of cysteine in distilled water. The solution can easily be prepared in your laboratory and filter sterilized, or the solution can be purchased from Applied BioPhysics (Electrode- stabilizing solution).

2. Allow the arrays to sit with the cysteine solution for a minimum of 30 minutes at room temperature. The time of the cysteine exposure depends upon the history of the array, for relatively newly etched arrays often the reaction is completed in only a few minutes, but to be certain the reaction has gone to completion, an hour-long exposure is recommended for all arrays.

3. Rinse the wells 3x in distilled sterile water and then fill with medium. Drying of the array should be avoided.

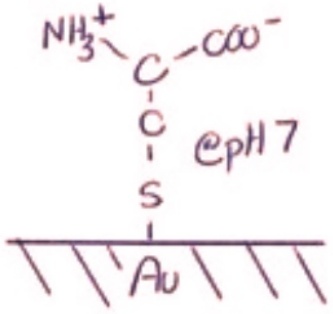

Figure 1. Sketch of cysteine binding to the gold surface of the electrode.

Electrical stabilization

An alternative way to clean the electrodes uses the ECIS® electrical stabilization method. This feature is built into the ECIS® software. One simply fills wells with complete medium, loads the ECIS® arrays onto the Array Station, clicks on the wells to be stabilized and then starts the process by clicking Stabilize in the ECIS® software. This process usually takes several minutes depending upon the number of wells treated and the condition of the electrodes– so please be patient. In addition, following electrical stabilization about 30 minutes equilibration is required for the electrodes to settle into their final impedance values. Waiting this period helps minimize impedance drift during an experimental run and establishes good reproducibility from one experiment to another.

Θ Note: This procedure applies a high field current across the electrodes and will thus negatively effect any cell on the electrode. Therefore this procedure should only be applied to cell free wells.