Applied BioPhysics

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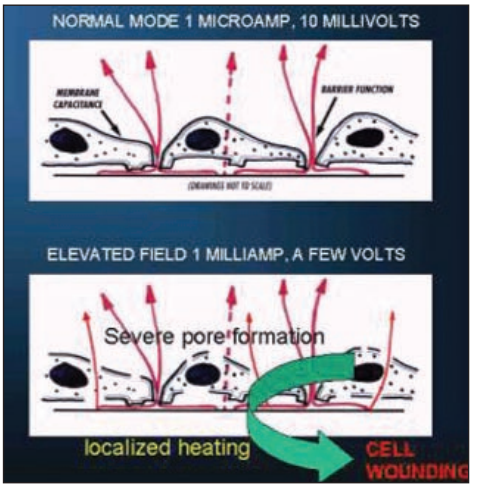
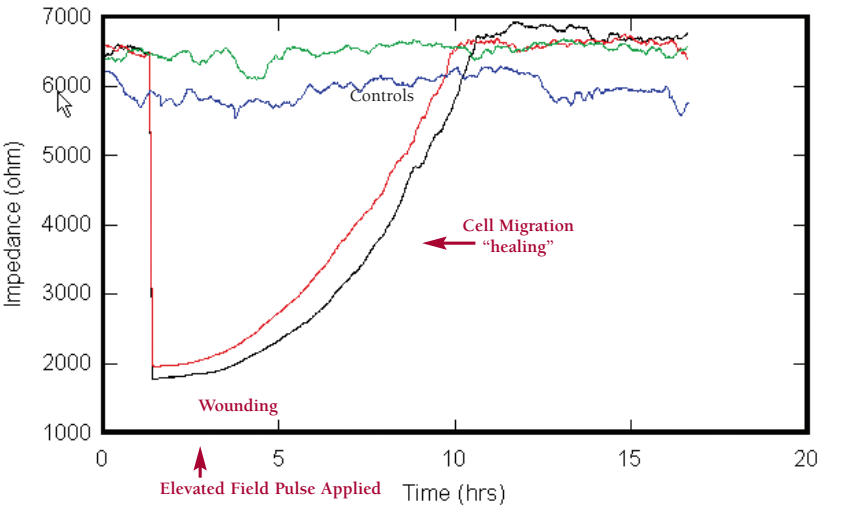
ECIS™ Electroporation / Wounding Module

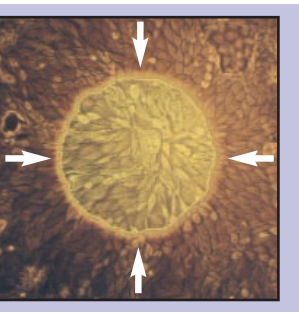




THE NEW ECIS ASSAY

ECIS has now been modified such that electroporation* and automated wound healing assays (Patent pending) can be performed. In normal ECIS measurements, a current of less than a microampere is used. This is undetected by the cells, and, in its measuring mode, ECIS essentially eavesdrops on cell behavior electrically. When the current is boosted 1000 fold to a milliampere, the resulting voltages across the cell membranes result in electroporation. If this is applied for only a few milliseconds, the cells recover, and it is possible to insert impermeable molecules including DNA constructs into the cytoplasm. When the high current is applied for several seconds, cell death ensues due to severe electroporation and possible local heating effects.

The ECIS wound is very well defined, as it includes only those cells on the 250 micrometer diameter electrode (shown below). Death can be verified both with the ECIS measurements and with vital staining.Typical ECIS data involving this assay is shown in the above figure. Here BSC1 cells were first grown as complete monolayers, and the impedance traces from four confluent wells can be seen on the graph. At the arrow, an elevated field was applied to two of the wells, wounding the cells on the small electrode and causing the impedance to drop to that of an open electrode. Over time these two traces return to control values, as the healthy cells outside of the small electrode migrate inward to repopulate the wounded area and replace their dead cohorts (healing). These types of data are highly reproducible and respond to culture conditions.



The figure to the left shows an ECIS electrode covered with MDCK cells 24 hours after the elevated field wounding took place. Note the subtle radial patterns indicated by the arrows as the cells have migrated inward.

This is a completely automated assays requiring a minimum of labor. Both cell wounding and measurements of the subsequent healing process are carried out under computer control without opening the door of the incubator.

* Joachim Wegener, Charles R. Keese and Ivar Giaever, BioTechniques 33(2), 348-357 (2002).

TRADITIONAL WOUND HEALING ASSAYS

Wound healing assays have been carried out in tissue culture for many years to estimate the migration and proliferation rates of different cells and culture conditions. These assays generally involve first growing a confluent cell monolayer. A small area is then disrupted and a group of cell destroyed or displaced by scratching a line through the layer with, for example, a needle. The open gap is then inspected microscopically over time as the cells move in and fill the damaged area. This "healing" can take from several hours to over a day depending on the cell type, conditions, and the extent of the "wounded" region.

Problems of reproducibility and quantification