Abstract: It has been observed that when certain electrical potentials are applied across a cell they can induce the formation of pores in the cell membrane and consequently increase the permeability of the cell to macromolecules. This phenomenon is known as electroporation. Since the first report on gene transfer by electroporation, it has become a standard method for introduction of macromolecules into cells. Currently, electroporation is normally done in batches of cells between electrodes and in organisms with electrodes inserted in the tissue. There is little control over the permeabilization of individual cells. Therefore, it is very difficult to study the fundamental biophysics of cell membrane electro-permeabilization, which is not yet understood, and to design optimal and reversible electroporation protocols for individual cells. Although the biophysics of electroporation are still not fully understood, indirect evidence shows that micro aqueous pores with diameters of tens to hundreds of angstroms are created in cell membrane due to the electrical field induced structural rearrangement of the lipid bilayer. It occurred to us that if electroporation induces pores in the cell membrane than, in a state of electroporation, a measurable current should flow through the individual cell. From this idea, we have developed a new micro-electroporation technology that employs a “bionic” chip to study and control the electroporation process in individual cells. The micro-electroporation chips are designed and fabricated using standard silicon microfabrication technology. Each chip is a three-layer device that consists of two translucent poly silicon electrodes and a silicon nitride membrane, which all together form two fluid chambers. The two chambers are interconnected only through a micro hole on the dielectric silicon nitride membrane. In a typical process, the two chambers are filled with conductive solutions and one chamber contains biological cells. Individual cells can be captured in the micro hole and thus incorporated in the electrical circuit between the two electrodes of the chip. When the cell is in its normal state no current flows through the insulating lipid bilayer and consequently between the electrodes. However, when the electrical potential across the electrodes is sufficient to induce electroporation, a measurable current will flow through the pores of the cell membrane and between the electrodes. Measuring currents through the bionic chip as a function of electrical potential will determine the potential that induces the electroporation. The chip behaves somewhat similarly to an electrical diode, with no current at potentials that do not induce electroporation and currents at potentials that induce electroporation.

The work leads to two items: The first item is the proof of our hypothesis that if electroporation produces aqueous channels in the membrane than when cells become electroporated they also should carry electrical currents, and otherwise they do not. This makes the process of electroporation instantaneously detectable when incorporated in an electrical circuit. The paper has demonstrated the hypothesis. This is a universal phenomenon, which can occur when the cells are outside or inside the body. Electroporation has been used in the past for introduction of gene constructs in cells in suspension and in cells in the body, for production of transgenic organisms or for treatment of genetic diseases. However there was no immediate feedback for knowing if the cell has actually been electroporated in response to the electrical potential or not. Now an electrical current measurement tells. The second item is the actual feat of producing the chip. The chip has immediate industrial application in introducing macromolecules in cells. With the ability to manipulate individual cells and detect the electrical potentials that induce electroporation in each cell, the chip can be used to study the fundamental biophysics of membrane electro-permeabilization on single cell level and in biotechnology, for controlled introduction of macromolecules, such as gene constructs, into individual cells.
Figure 1. Schematic cross section of a micro-electroporation chip

Figure 2. A human prostate adenocarcinoma cell (ND-1) being trapped in the micro hole
Figure 3. The current-voltage pattern of reversible electroporation of a human breast cell (HBL) captured in a 4um hole device under 60ms square pulses.
(Top curve: for open hole, middle: for cell plugging the hole, bottom: for closed hole)

Figure 4. The current-voltage pattern of irreversible electroporation of a human prostate adenocarcinoma cell (ND-1) captured in a 2um hole device under a 640ms ramp pulse.

1: applied voltage between two electrodes
2: current curve for cell plugging the hole
3: current curve for open hole mode
4: current curve for close hole mode


