Electroporation

Electroporation is the use of a transmembrane electric field pulse to induce microscopic pathways (pores) in a bio-membrane. These pores are sometimes called "electropores." Their presence allows molecules, ions, and water to pass from one side of the membrane to the other. As the right side bar shows, the electropores are located primarily on the surfaces of cells which are closest to the electrodes. If the electric field pulse has the proper parameters, then the "electroporated" cells can recover (the electropores reseal spontaneously), and the cells can continue to grow. The time for the pathways to form is about one microsecond. The time to reseal is minutes.

The use of electroporation was described by Neumann in the early 1980s. The routine use of electroporation became very popular with researchers through the 1980s because it was found to be a practical way to place drugs, or other molecules into cells. In the late 1980s, scientists began to use electroporation for applications in multicellular tissue.

In the early 1990's Lluis Mir of the Institute Gustave-Roussy was the first to use electroporation in a human trial to treat external tumors.

Research has shown that the induction of electropores is affected by three major factors. First, cell-to-cell biological variability causes some cells to be more sensitive to electroporation than other cells. Second, for electropores to be induced, the product of the pulse amplitude and the pulse duration has to be above a lower limit threshold. Third, the number of pores and effective pore diameter increase with the product of "amplitude" and "duration." Although other factors are involved, this threshold is now understood to be largely dependent on a fourth factor, the reciprocal of cell size. If the upper limit threshold is reached pore diameter and total pore area are too large for the cell to repair by any spontaneous or biological process. The result is irreversible damage to the cell or cell lyses. Because the mechanism of electroporation is not well understood, the development of protocols for a particular application has usually been achieved empirically, by adjusting pulse parameters (amplitude, duration, number, and interpulse interval).

Early research on electro-pore-mediated transport across membranes assumed that
simple thermal motion (i.e. diffusion) propelled molecules through electropores.

Research in the late 1980s and early 1990s showed that certain experimental conditions and parameters of electrical pulses may be capable of causing many more molecules to move per unit time than simple diffusion. For example, there is good evidence (Dimitrov and Sowers, 1990) [1] that molecular flow is in the direction of the arrow in the sidebar but there is also good evidence (Sukharev, et al., 1992) [2] that DNA movement is in the opposite direction of the arrow in the sidebar. This implies that electroporation has polarity dependence. Although this apparent contradiction will have to be resolved by future basic research, it clearly suggests that pulse generators with polarity-adjustable electrical parameters are necessary for protocol development.

An additional important consideration is that during the electroporation pulse, the electric field causes electrical current to flow through the cell suspension or tissue. Biologically relevant buffers for cells, bathing media, and fluid in extra-cellular space in tissues contain ionic species at concentrations high enough to cause high electric currents to flow. These currents can lead to dramatic heating which is biologically unacceptable. Principles of physics suggest that the early part of exponentially decaying pulses does most of the membrane porating but the late part continues to heat the medium.

There have been two main waveform categories of "porating" pulses: exponentially decaying, and rectangular (square) wave. These waveform qualities were a matter of customary electrical engineering principles and the fact that pulse generators designed for one waveform usually could not deliver the other waveform. Moreover, only a few side-by-side studies were conducted which showed a fundamental and universal superiority of one waveform over another. In cases where there is evidence that an exponentially decaying pulse may have an advantage for a particular application, a protocol which delivers two pulses, one which is high in amplitude and short in duration followed by a second which is low in amplitude but long in duration, may simulate the effects of the exponentially-decaying pulse or even provide an improved result. Indeed, the PulseAgile® capability of Cyto Pulse pulsers has more pulse flexibility than any other currently available instruments.

References, General Electroporation

Books


Journal Articles


Klenchin VA, Sukharev SM, Chernomordik LV, Chizmadzhev YA, Electrically induced DNA uptake by cells is a fast process involving DNA electrophoresis, 1991, Biophys J. 60:804-811


