Abstract

Three miniaturised electroporation and electroinjection methods were developed to introduce exogenous compounds into single liposomes, and single cells, as well as small populations of cells in organotypic tissues, and tissues *in vivo*.

The first method was developed for electroporation of single surface-immobilised cells using solid carbon fibre microelectrodes, 5 μm in diameter. The protocol was characterised by patch-clamp recordings and fluorescence microscopy. From transmembrane current responses, pore open times, as well as pore opening, and closing kinetics were determined. From both patch clamp, and fluorescence measurements, the threshold transmembrane potential for electroporation was determined to be ~250 mV.

A second approach for electroporation was developed based on using an electrolyte-filled capillary (EFC) made of fused silica (30 cm long, 375-μm o.d. 30-50-μm i.d.). A DC voltage pulse (square wave, duration 5-60 seconds) induced pore formation and the electroosmotic flow in the EFC delivered the cell-loading agent to the site of pore formation. The threshold transmembrane potential for electroporation was determined to be ~85 mV by patch clamp and fluorescence measurements. The method was used to introduce the DNA-intercalating dye YOYO-1 to single cell processes, single cell somas, small populations of cells in organotypic tissues, and tissues *in vivo*. The enzyme substrates fluorescein diphosphate (FDP) and casein BODIPY FL were introduced to single cells for detection of alkaline phosphatase and proteases, respectively. Detection of intracellular receptors (IP_3 and ryanodine) was performed by introduction of selective receptor agonists and blockers. Electroporation of populations of cells in microwells (100 x 100 x 45 μm) fabricated in poly (dimethylsiloxane) was demonstrated by introduction of FDP.

Third, an electroinjection method for introduction of materials into single cells and liposomes was developed. This method uses a combination of electrical and mechanical forces to penetrate the lipid bilayer membrane. A DC electric field of 10-40 V/cm, and 1-10 ms pulse duration was applied with a carbon fibre microelectrode (5 μm in diameter) in combination with an injection tip (2 μm o.d.) filled with the loading agent. Injection was performed by pressure. Small sample volumes (5-500 x 10^{-15} L) of stained DNA, and colloidal particles (30-200 nm in diameter) could be electroinjected to single liposomes and cells. Selective introduction of colloidal particles to different sub-compartments of cells was also accomplished.

The methods developed can be used to manipulate cell properties, even at the subcellular level. Manipulation, sensing, transfection, probing of intracellular pathways, phenotype profiling, and screening are examples of applications, which may have an impact on the understanding of intracellular chemistry, and for single-cell analyses. These methods might be useful in areas such as drug discovery, proteomics and for the pharmaceutical industry.

KEYWORDS; Capillary electrophoresis, single-cell, electrolyte-filled capillary, fluorescence, patch clamp, receptors, electroporation, electroinjection, intracellular proteins, liposome.