

## Abstract

A miniaturized electroporation method was developed for introduction of membrane-impermeant substances, such as dyes, DNA, RNA, peptides, proteins as well as other polar or charged species into single cells and giant liposomes (1-to-100  $\mu\text{m}$  in diameter). This technique is based on the electric-field-induced permeabilisation in bilayer membranes, using a low-voltage pulse generator and 5- $\mu\text{m}$ -diameter carbon fiber electrodes. Typically, electric field strengths of 1-3 kV/cm (dc, rectangular waveform) and 1 ms duration were used in single-cell electroporations. The high spatial resolution was demonstrated by the introduction of polar cell-impermeant molecules, such as fluorescein, Fluo-3 and cDNA (p-RAY1, GFP-expressing reporter gene), to single progenitor cells derived from adult rat hippocampus as well as to single COS-7 cells, and by electroporation of submicrometer-sized cell-processes of progenitor cells. Preliminary results show that organelles were electroporated inside cells at higher electric field strengths.

The same experimental setup was used for pairwise cell-cell electrofusions. Fusion was induced by application of electric field strengths of about 2-3 kV/cm (dc, rectangular waveform) and 1 ms duration. Homokaryons of Jurkat, PC 12, COS 7, and NG 108-15 cells, as well as heterokaryons of PC 12 and NG 108-15 were formed, thereby allowing alterations of the surface properties, cytoplasmic, and genetic contents of cells. These properties, except for genetic, could also be manipulated by fusing single giant liposomes to NG 108-15 cells.

A prototype microfluidic device was developed for pairwise fusion of selected fusion partners (single cells or liposomes) in a sequential manner. Using this device a large number of combinatorially synthesized liposomes with complex compositions and reaction systems can be obtained from a small set of starting liposomes or cells. Cells or liposomes were separated in different chambers interconnected by 3-mm-long glass capillaries (10-30  $\mu\text{m}$  i.d., 30-100  $\mu\text{m}$  o.d.). Fusion partners were individually transferred using a combination of optical trapping and microfluidic device translation into a fusion chamber for electrofusion.

An electroinjection method was developed for the introduction of colloidal particles (30- to 200 nm in diameter), small uni- and multilamellar liposomes, and biopolymers into cellular and liposomal interiors. Electromechanical penetration of cell and liposome membranes with a  $\sim 2$   $\mu\text{m}$  o.d. micropipette tip filled with the material to be injected was obtained by the application of dc electric fields of 10-40 V/cm and 1-10 ms duration, in combination with mechanical pressure. Small-sized samples, 50-500  $\times 10^{-15}$  L, could be injected to liposomes. YOYO-1 stained T7-phage DNA (radius of gyration,  $R_G = 0.56$   $\mu\text{m}$ ) was injected into the cytosol and nucleus, respectively of single PC 12 cells. By this technique it is also feasible to construct complex artificial cells with multiple reaction systems and synthetic organelles, for studies of, for example, intracellular signalling systems with components that translocate between different compartments.

Electroporation, electrofusion, and electroinjection protocols were developed to initiate and perform chemical reactions in  $\geq 0.5$  fL biomimetic liposome containers. Typically, product formation was monitored using far-field fluorescence microscopy. For example, reactions between T2-phage DNA ( $R_G = 1.1$   $\mu\text{m}$ ) and YOYO-1 were initiated by dual electroinjections into a single giant unilamellar liposome. Using electrofusion, one  $\text{Ca}^{2+}$ -containing liposome and one Fluo-3-containing liposome were combined to initiate a chelation reaction. An enzyme-catalyzed reaction, the two-step hydrolysis of fluorescein-diphosphate to fluorescein by alkaline phosphatase, was monitored using single-molecule-sensitive confocal fluorescence microscopy inside 1- $\mu\text{m}$ -diameter liposomes. The ultrasmall characteristics of the biomimetic reactors permit the study of fast chemical kinetics due to short mixing times.

**KEY WORDS:**Electrofusion, electroporation, electroinjection, lipid, membrane, bilayer, liposome, transfection, fluorescence, biomimetic, reactor, microfluidics, microelectrode, permeabilization, DNA, dye