La Prensa Medica

Short Communication

A SCITECHNOL JOURNAL

Single cell nano-electroporation to laser induced photoporation: Novel approaches for cell therapy and diagnostics

Tuhin Subhra Santra

Abstract

The ability to precisely deliver of foreign cargo into single living cells is of great interest in cell biology and therapeutics research. Conventional bulk electroporation is widely used but has been known to cause high percentage of cell death and require high voltage sources. Microfluidic electroporation platforms can provide high delivery efficiency with high cell viability through better-controlled electric fields applied to cells. Here we develop micro/nano fabricated single cell electroporation platforms, which is an efficient and fast method for multi-nanolocalized single cell nanoelectroporation, where electroporation takes place on a multiple region of individual single cell membrane using ITO nano-electrodes array. The gap between two nanoelectrodes are 70 nm with triangle tip diameter of 40 nm, which intense an electric field in a precise region of single cell membrane to deliver biomolecules with high transfection efficiency and high cell viability. On the other hand we developed photoporation based devices, where nano-second pulse laser is used to interact with metal or metal nanoparticles and form plasmonic nanobubbles, which rapidly grew, coalesced and collapsed to induce an explosion, resulting strong fluid on the cell membrane. Thus plasma membrane can disrupt and form transient membrane pores, allowing the delivery of cargos from outside to inside the cell. Using both of these techniques we successfully deliver dyes, DNA, RNA, QDs and nanoparticles, bacteria in cancer cells as well as stem cell. These new approaches can allow us to analyse different dyes/biomolecules interaction in single living cell with spatial, temporal, and qualitative dosage control, which potentially applicable for medical diagnostics and therapeutic studies.

Introduction

The faculty to precisely distribute of peregrine cargo into single living cells is of great interest in cell biology and therapeutics research. Conventional bulk electroporation is widely used but has been kenned to cause high percentage of cell death and require high voltage sources. Microfluidic electroporation platforms can provide high distribution efficiency with high cell viability through better-controlled electric fields applied to cells. Here we develop micro/nano fabricated single cell electroporation platforms, which is an efficient and expeditious method for multi-nanolocalized single cell nanoelectroporation, where electroporation takes place on a multiple region of individual single cell membrane utilizing ITO nano-electrodes array. The gap between two nanoelectrodes are 70 nm with triangle tip diameter of 40 nm, which profound an electric field in a precise region of single cell membrane to distribute biomolecules with high transfection efficiency and high cell viability.

On the other hand we developed photoporation predicated contrivances, where nano-second pulse laser is utilized to interact with metal or metal nanoparticles and form plasmonic nanobubbles, which expeditiously grew, coalesced and collapsed to induce an explosion, resulting vigorous fluid on the cell membrane. Thus plasma membrane can disrupt and form transient membrane pores, sanctioning the distribution of cargos from outside to inside the cell. Utilizing both of these techniques we prosperously distribute dyes, DNA, RNA, QDs and nanoparticles, bacteria in cancer cells as well as stem cell. These incipient approaches can sanction us to analyse different dyes/biomolecules interaction in single living cell with spatial, temporal, and qualitative dosage control, which potentially applicable for medical diagnostics and therapeutic studies. Keywords: Electroporation, electropermeabilization, transfection, single cell, microfluidic probe, nanofountain probe NFP System: For genuine time monitoring of live cells, during electroporation, an inverted fluorescence microscope is employed. Adherent cells are cultured on a coverslip coated with a conductive thin film, e.g., Cr/Au, and placed in a liquid cell (Park Systems, CA) on the microscope sample stage. Once a target cell is optically culled, the NFP probe is displaced, utilizing a nanomanipulator, such that the NFP tip covers the cell in a region of interest. SCEP: Models of the NFP-E system indicate that the electric potential drop through each probe is independent, parallelized electroporation with multiple probes does not require higher input voltage than single probe electroporation, the transmembrane electric potential drop increases with larger input voltage and smaller gap between the NFP tip and cell membrane, the NFP creates a highly focused electric field only within a small region of interest, and local voltage at the tip is much smaller than the input voltage. To validate such predictions, we performed single cell electroporation experiments on HeLa cells using the NFP-E system. We obtained HeLa cells from the American Type Culture Collection (ATCC #CCL-2) and cultured them in Dulbecco's Modified Eagle Medium (SIGMA) with L-glutamine and phenol red as pH indicator, supplemented with 10% FBS (SIGMA) and 1X penicillin/streptomycin (SIGMA). The cultured cells were maintained in a humidified incubator at 37 °C and 5% CO2. On the day of the experiment, the coverslip with plated cells was rinsed multiple times with DMEM without phenol red to avoid autofluorescence during fluorescence imaging of the cells.

As mentioned earlier, potential drop through each probe on a NPF chip is independent of the other probes; therefore, each probe can be used interchangeably during single cell electroporation. We experimentally confirmed the theoretical prediction. For example, we observed that a NFP probe was clogged after continuous use due to repeated interaction between probe and cells. Even when a particular probe was clogged, we could continue electroporation by switching to another probe on the same NPF chip without modifying any of the electrical input signals. The multiple parallel probes are a unique advantage of the NFP-E system in comparison to other microscale electroporation methods, e.g., micropipette based electroporation.

***Corresponding author:** Tuhin Subhra Santra, Indian Institute of Technology Madras, India