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

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Abstract

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. The onset of contact is detected either by optical optical discernments of the cell morphology or by the transmutation in electrical resistance due to the sealing between probe tip and cell. After contact, an electric pulse is applied between the Ag/AgCl wire and the conductive coverslip to induce electroporation. The external input signal is engendered by a pulse engenderer (Standard Research Systems DS345), amplified by a voltage amplifier (OPA445, Texas Instruments), and monitored utilizing an oscilloscope (LeCroy 9384L). The electrical resistance of the circuit during electroporation is monitored utilizing a digital multimeter (Agilent 34401A). Further details on the experimental procedures are given in a subsequent section

SCEP

Models of the NFP-E system indicate that (1) the electric potential drop through each probe is independent, (2) parallelized electroporation with multiple probes does not require higher input voltage than single probe electroporation, (3) the transmembrane electric potential drop increases with larger input voltage and smaller gap between the NFP tip and cell membrane, (4) the NFP creates a highly focused electric field only within a small region of interest, and (5) 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. For electroporation experiments, the cells were plated the day before the experiment on a round 25 mm glass coverslip with a thin Cr/Au film and incubated in DMEM media. The thin metal film acts as one of the electrodes in electroporation experiments. The thickness of the coating was chosen to ensure both low resistance and good transparency for imaging cells using an inverted optical microscope. 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. The coverslip was then placed in a liquid cell (Park Systems) and imaged using the inverted optical microscope, while

DMEM media without phenol red was added to maintain the cells submerged throughout the electroporation experiments.

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.

Conclusion

A robust and nondestructive method for controlled, in situ distribution of molecules into cells is needed to advance the state-of-the-art in personalized medicine and therapeutics. Development of SCEP instrumentation like the NFP-E system, and protocols for practical use in biotechnology research, drug revelation, and personalized therapeutics, could transform the future of these fields. Hence, demand is great for the development of an ecumenical implement for single cell electroporation that is robust, facile to utilize, efficient, and gentle to cells.

Bulk electroporation is increasingly being utilized as the transfection method of cull despite being profoundly disruptive to cells due to astronomically immense heat generation from the kV-range applied voltage. In additament to toxicity, the bulk electroporation technique withal suffers from issues such as lack of dosage control because the uptake of biomolecules after pore generation is governed by desultory diffusion, resulting in a heterogeneously transfected cell population. Thus, the technique is not felicitous for applications involving sensitive cells (e.g., primary cells) that require high yield with precise cellular distribution (dosage). In contrast, we have demonstrated that the NFP-E system is minimally disruptive to cells, only a diminutively minuscule portion of the cell membrane is subjected to the electric field and probe-membrane contact can be detected electronically, so efficacious transfection was accomplished with applied input voltages of only ~30 V leading to transmembrane voltages Vm~0.6 V. Moreover, we demonstrated the competency to precisely control and monitor the contact force applied by the probe on the cell, utilizing optical imaging and electrical detection, truncates stress and potential cell damage upon contact. Another advantage of NFP-E is its compatibility with mundane microscopy methods such as AFM [22] as well as epifluorescence and confocal microscopy, which sanctions the entire transfection process and post-transfection cellular replication to be monitored in an optical microscope. Further advantages include the diminutive volume of each NFP microchannel (~3 pL) and the precise control of the distribution of these often sumptuous biomolecules or other agents. Because the molecules are confined in the NFP, the engendered nanopores are exposed to a high concentration of transfection agent, minimizing the amplitude of consumable biomolecules compared to other methods. In additament, the NFP chips are fabricated at the wafer level so batch processing can be yarely scaled up for mass engenderment.

We have demonstrated here electroporation of single cells utilizing NFP technology for the distribution of membrane-impermeable biomolecules into HeLa cells. The NFP-E system has unprecedented capabilities for targeted transfection such as single cell selectivity, high transfection efficiency, dosage control, and ultrahigh cell viability. The NFP-E implement has potential to enable novel biological studies including: (1) single cell analysis (gene expression studies, time-dependent cell biology, protein interaction studies, drug toxicity and replication), (2) cell line development, and (3) stem cell reprogramming/differentiation.