IN-SITU SINGLE CELL ELECTROPORATION USING OPTOELECTRONIC TWEEZERS

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ABSTRACT

Optoelectronic Tweezers are used to achieve light-induced, *in-situ* electroporation of HeLa cells. By controlling electrical bias, patterned light induces either single cell movement or electroporation. Fluorescent dye and dielectrophoretic response are used to monitor electroporation.

INTRODUCTION

Electroporation is a common tool for applications including DNA transfection, drug delivery, and gene therapy. During electroporation, cells are subjected to electric fields which cause the creation of nanometer-scale pores in the cell membrane allowing fluid flow from the medium into the cytoplasm. However, the two most common electroporation techniques employed today are limited either by cell selectivity or small throughput. Much work in recent years has tried to ameliorate these issues by moving towards a microscale electroporation platform [1, 2]. However, these techniques do not provide both the advantage of high throughput and single cell selectivity. Here we report the use of Optoelectronic Tweezers (OET) to achieve *in-situ* electroporation of HeLa cells. Due to the dynamic nature of OET, single cells can be selected, manipulated, and then electroporated in parallel.

DEVICE OVERVIEW

Optoelectronic tweezers uses patterned light to alter the conductivity of a photosensitive film to create localized electric field gradients. These gradients result in a dielectrophoretic (DEP) force on particles in the vicinity. Because of the low light power necessary for actuation, compared to the more traditional optical tweezers, thousands of simultaneous traps can be created and manipulated in parallel [3].

The OET device with optical setup is schematically drawn in Fig. 1. It consists of a piece of ITO-coated glass with a 1 μm layer of photosensitive a-Si:H deposited via plasma enhanced chemical vapor deposition. A top piece of ITO-coated glass is used, in addition to the bottom piece, to sandwich a solution containing the particles of interest. An AC bias is applied between the two ITO electrodes and light patterns are supplied via a computer controlled projector focused through a 20x objective. Observation occurs from the topside with a CCD camera.

Cells are first selected and positioned using the projected light as reported elsewhere [4]. Next, the cells are subjected to an electroporation bias which causes temporary breakdown of the cell membrane to occur. This allows nano-tags (e.g. molecules, nanoparticles) to enter the cell. The porated cells are then moved once again by returning to the normal OET bias and altering the light pattern.

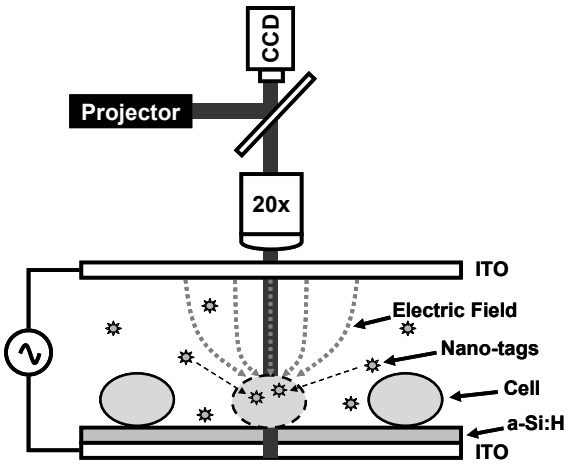

Fig. 1: Overview of OET electroporation platform. Patterned light localizes electric field across a cell of interest resulting in selective electroporation and allowing nano-tags (e.g. molecules, nanoparticles) to enter the intracellular matrix.

MODELING

The DEP force is proportional to the Claussius-Mossotti (CM) factor and the gradient of the square of the electric field. In order to model the expected DEP force on cells, a multi-shell model is used to approximate the CM factor [5]. As shown in the inlay of Fig. 2, this consists of the cell membrane, cytosol, nuclear membrane, and nucleoplasm. This factor is then multiplied by the gradient of the square of the electric field which is extracted by simulating the OET device in a commercially available finite element modeling package (Comsol 3.2a). Using typical cellular parameters, a normalized frequency response of the OET device acting on HeLa cells is shown in Fig. 2. In this figure, we plot the relative DEP force for HeLa cells with varying cytosol and nucleoplasm conductivities. The conductivity varies from that of the cell (0.53 S/m) to that of the experimental medium (10 mS/m). As one can see, the DEP force switches from positive to negative in the kilohertz range as the conductivity is decreased.

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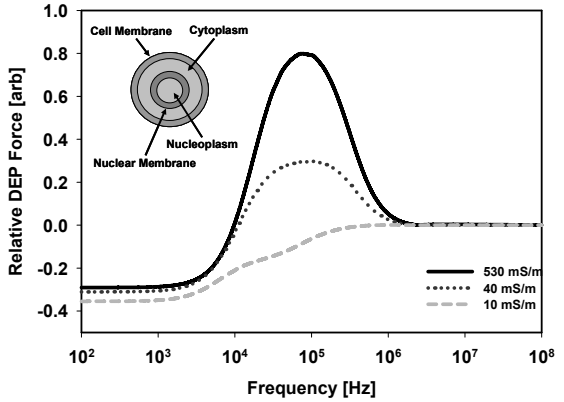

Fig. 2: Relative DEP force for cells with varying cytoplasmic and nucleoplasmic conductivity. As conductivity decreases, DEP force switches from positive to negative over the kHz frequency range. Inlay shows the cell model used for calculating the Claussius-Mossotti factor taking into account both cell and nuclear membrane.

EXPERIMENTAL RESULTS

HeLa cells were suspended in a commercially available electroporation medium (Cytoporation Medium T, Cyto Pulse Sciences, Inc.) with a conductivity of 10 mS/m and density of 1.1 million cells per milliter. Propidium Iodide (PI) was added at a concentration of 1:500 (PI:solution). PI is a membrane impermient dye which fluoresces red only in the presence of nucleic acids. A 1 μL droplet of solution was then placed in the OET device. A 7 Vppk, 100 kHz bias was then applied. When a light pattern (~2 W/cm^2) illuminates the cell of interest, it results in a positive DEP force with nominal dye uptake, as evidenced in Fig. 3 and 4. Next, a 5 second 12 Vppk, 100 kHz bias is applied to the cell. This causes substantial uptake of PI by the cell indicating electroporation. However, the cell still exhibits a positive DEP response. This indicates that the electroporation process has not changed the cell's interior conductivity substantially. Lastly, a 5 second 15 Vppk, 100 kHz signal is applied which results in continued uptake of PI by the cell. However, this time the cell exhibits a negative DEP response following the voltage stimulus. This is consistent with the conductivity of the cytosol and nucleoplasm decreasing to that of the surrounding medium as shown in Fig. 2. This further indicates that electroporation of the cell has occurred.

CONCLUSION

The successful light-induced, *in-situ* electroporation of individual HeLa cells has been achieved using Optoelectronic Tweezers. The extent of the electroporation of the cell is controlled by varying the applied voltage. The applied voltage controls how much fluid is exchanged across the cell membrane resulting either positive or negative DEP response. This approach can easily be parallelized to achieve a high throughput electroporation assay with single cell selectivity.

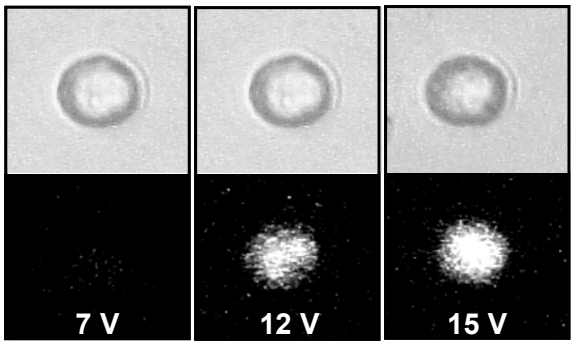

Fig. 3: Bright field (top) and fluorescent (bottom) images of a HeLa cell subjected to 7 Vppk, 12 Vppk, and 15 Vppk at 100 kHz for 5 seconds.

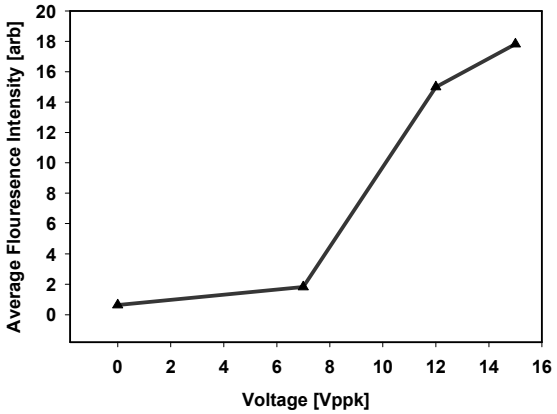

Fig. 4: Average fluorescent intensity of a HeLa cell for varying voltages.

ACKNOWLEDGMENT

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