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On-Chip Electroporation: Characterization, Modeling and Experimental Results

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***Abstract*—In this paper, we describe an on-chip electroporation (EP) method for high precision nano-injection of bio-molecules into single cells. EP is an electrical stimulation method to create nano-pores on the cell plasma membrane. Herein, we first put forward the computational models of the cultured cells microelectrodes. We thereafter discuss practical considerations by demonstrating the preliminary experimental results. The mouse fibroblast cells are cultured above electrodes while experiencing a low frequency (10 Hz) electrical field (EF) in the presence of propidium iodide (PI).**

I. INTRODUCTION

On-chip electroporation (EP) methods have recently attracted the attention of researchers for high transfection efficiency of biomolecules into cells for gene therapy or cell reprogramming purposes [1-2]. A microelectronic platform can efficiently be employed to control the electrical stimulation and consequently control of the biomolecule injection into living cells. As shown in Figs. 1, the living single cells can be cultured on the planar electrodes realized above a microelectronic chip incorporated with microfluidics. A bio-adhesive material such as gelatin is used to coat microelectrodes (MEs). The created pores on each cell (Fig. 1b) allow the biomolecules to diffuse into cells.

A microelectronic chip offers several advantages for high throughput single cell EP using integrated sensors and actuators based on capacitive, Ion-Selective-Field-Effect-Transistor (ISFET), impedometric, magnetic and dielectrophoresis techniques [3]. The transfection of bio-molecules into cells can be performed mostly using viral or electro-transfection methods [4]. As shown in Fig. 1b, the application of electrical field (EF) creates pores and results in higher diffusion of bio-particles in living cells. By removing EFs, the cells become normal. EP is a non-invasive method if the EFs near the cells do not exceed a threshold voltage. The trans-membrane voltage can be expresses as shown in equation (1) [5].

$$\Delta\Phi = \frac{3}{2}\frac{\sigma_e[3dR^2\sigma_i + (3d^2R - d^3)(\sigma_m - \sigma_i)]}{\Theta} ERCos\ \theta$$

$$\Theta = [R^3(\sigma_m - 2\sigma_e)(\sigma_m - 0.5\sigma_i) - (R-d)^3(\sigma_e - \sigma_i)(\sigma_i - \sigma_m)] \quad (1)$$

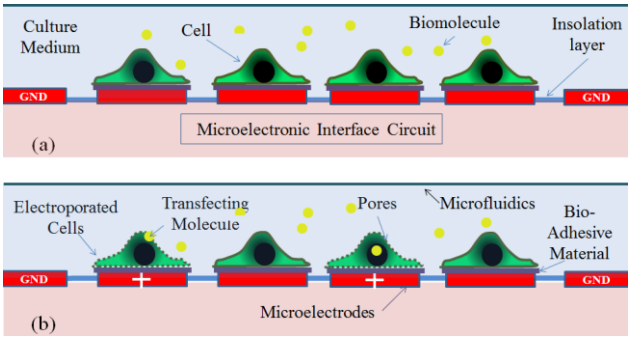

Figure 1: On-chip EP: the cultured cells on MEs realized on top most metal layer of microelectronic chips, prior to (a) and after (b) electrical stimulation.

Where $\Delta\Phi$, R and E are the trans-membrane potential, radius of the cell and applied EF respectively. Also, θ defines angle between electric field lines and a normal vector from cell's center up to measurement point. In this equation conductivities of different parts have been taken into account. σ_i,σ_m and σ_e represent conductivity of cytoplasm, membrane and external solutions. Assuming a spherical cell with thin high resistivity membrane, $\Delta\Phi$ can be expressed as follows [6]:

$$\Delta\Phi = 1.5 \cdot E \cdot R \cdot Cos\ \theta \quad (2)$$

The electrical field (E) adjacent to cell membrane is a function of several factors. These factors include the geometry of planer MEs, cell-electrode distance, pulse amplitude and duration, and the electrical properties of culture medium as well as living cells. Many papers have already reported the advantage of planar MEs for EP of living cells [7-9]. In all these remarkable works, the living cells are in contact with MEs. We believe that the performance of EP using planar MEs is affected by the distribution and layers' number of living cells cultured on MEs. The bio-molecules can also be injected into flowing cells directed in microfluidic channels integrated into MEs [10].

In the remainder of this paper, we first put forward the computational and experimental methodologies, then the results are demonstrated and discussed in section III followed by a conclusion in section IV.

II. METHODS AND MATERIALS

In this section, we briefly describe the computational and experimental methods to study the effect of cell culture conditions on the EP of bio-molecules into living cells.

A. Simulation method

In order to study the role of cellular confluency and distribution on the performance of EP, an Interdigitated Electrodes (IEs) array (see Fig. 2b) is modeled using Comsol Multiphysics. In this study, multilayers of fibroblast cells ($\varepsilon \cong 1$ and $\sigma \cong 5$ *S/m*) are cultured on this gold ME array with full coverage. A culture medium solution ($\varepsilon=0.1$ and $\sigma=42$ S/m) is used respectively. Herein, the generated non-uniform EF on cultured cells is simulated and the practical considerations are discussed in comparison with experimental results. The EF intensity in the edges of MEs is simulated and the role of edge effect in EP is studied experimentally.

B. Experimental Set-up

The experiments can be performed on micro-fabricated gold electrodes (Fig. 2a) underneath of cultured fibroblast cells. A sputtering method was employed to deposit thin titanium and gold layers with the thicknesses of 20 and 200 nm respectively on glass substrate. An array of IEs is used to study the cells cultured under various conditions. As shown in Fig. 2b, the glass substrate including several IEs bonded with a PDMS sheet. A hole is created on this sheet for cell culture purposes (pinkish color in Fig. 2b). Epoxy conductive glue is also used to connect the pulse generator to the gold bonding pads. We used this simple set-up for the preliminary experiments shown in the next section. In order to study the role of applied pulse duration and amplitude, another array of eight gold IEs is realized on glass substrate and bonded with PDMS microfluidic chambers (Fig. 2c). This glass substrate is attached to a printed circuit board (PCB) while the electrical input/output pads interconnected to the electrically conductive pads on PCB using epoxy conductive glue covered with non-conductive glue. A series of switches is also used to multiplex the IEs manually for applying the input signals.

C. Biological protocol

The mouse fibroblast cells are cultured on MEs while the surface of substrate including the electrodes coated with gelatin for better cell-surface adhesion. The culture medium is the mixture of 500ml of DMEM (4500 glucose high, glutamine, pyruvate), 50mls of FBS 1 mM Pyruvate, 2mM L-glutamine, 100 U/ml Penicillin, 100 µg/ml Streptomycin, 50µg/ml Gentamicin and 2.5µg/ml Amphotericin B. The detachment of cells is also performed using the conventional Trypsin protocol for the control of EP performance and further analysis. Propidium iodide (or PI) is a small DNA stain (668.4 Da) diffusing into living cells under EF. The transfection of PI fluorescence molecules stains the cells nucleus converting into red when excited with 488 nm wavelength light. PI molecules are used to control the EP efficiency.

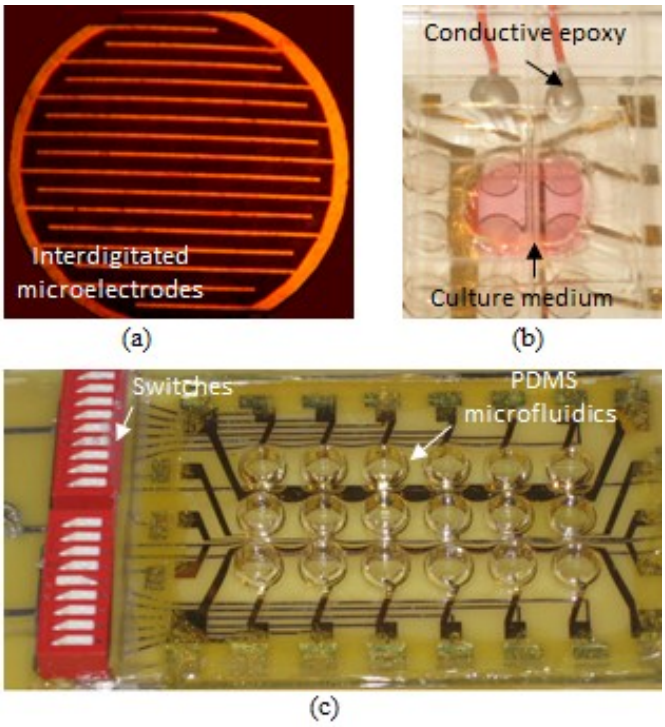

Figure 2. Micro-fabricated EP chips: (a) an IE, (b), PDMS sheet bonded on an array of IEs, (c) an array of electrodes bonded on microfluidic chamber array and attached on PCB.

III. RESULTS

In this section, we demonstrate the computational and experimental results while further discussions on these results will be put forward in the next section.

A. Computational results

In order to demonstrate the role of cells' placement and distribution on the EP efficiency, certain numbers of cell layers are located on top of MEs. Fig. 3 shows the EF generated by MEs in the presence of 0, 1, 3, 6 and 11 layers of cells cultured above electrodes. Based on these COMSOL simulation results, the higher number of cell layers, the higher EF intensity is generated near the electrodes and bottom layers.

As seen in Figs. 3a-3d, the living cells which are located near the edges of electrodes receive maximum intensity of the generated non-uniform electric field. By increasing the number of layers the EF intensity in the edges will also increase. This fact has also been highlighted in Fig. 4. In this figure, the average of electrical field intensity dropped on each layer is calculated when 1, 2, 3, 4, 5, 6 and 11 layers of cells are modeled above the electrodes. It is evident that the bottom layers experience higher EF intensity if the numbers of cell layers are increased. It is worth to mention that these simulation results are performed based on the assigned geometry of electrodes, electrical properties of culture medium and the applied electric potential (1volt).

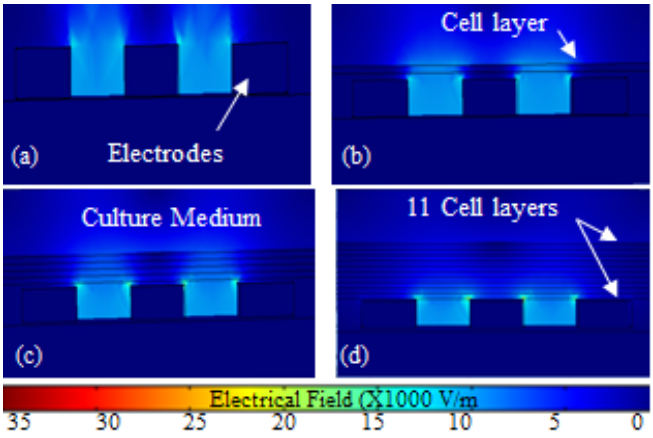

Figure 3. COMSOL simulation of EF (X1000 V/m) generated by IEs with on top: (a) culture medium, (b) a cell layer and culture medium, (c) six cell layers and culture medium and (d) eleven cell layers and culture medium.

The generated electrical currents due to the applied electrical voltage in between the electrodes can be seen in Table I. The higher current results in the higher electrochemical byproducts which might not be suitable for EP transfection of cells. Based on this table, top and bottom layers experience low and high electrical currents respectively.

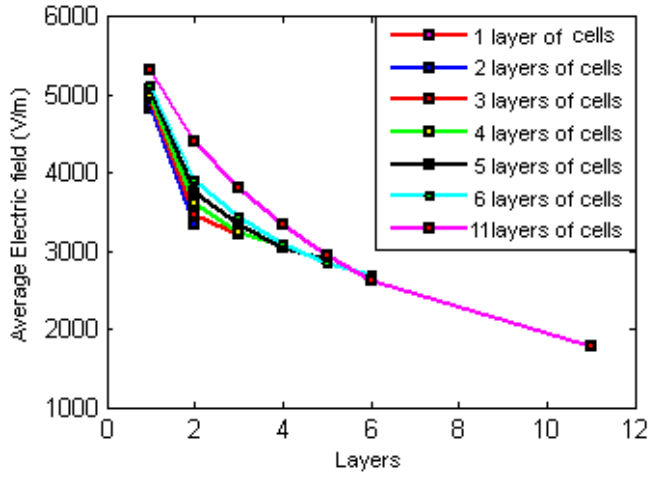

Figure 4. The EF dropped on each cell layer where 1, 2, 3, 4, 5, 6 and 11 layers of cells cultured on top of electrodes.

Therefore, it can be concluded that the top most layers of cells are less electroporated due to low electrical intensity in particular in the case of higher number of cell layers cultured above the electrodes. As the top most layers are exposed to transfecting molecules (e.g. for gene therapy or cell reprogramming), these cells layers can only be electroporated in a sufficient EF intensity. Fig. 5 demonstrates the variation of EF from cell cytoplasm to culture medium. This variation of EF across the cell membrane with only a few nanometers thickness (<15nm) corresponds to trans-membrane voltage which is responsible for EP. Actually, the differential voltage across the membrane, which increases the conductivity of cell membrane due to creation of nano-scale pores, can be approximately be calculated using these simulation results.

Table I: Average current density (A/m^2)for different layers.

	L-1	L-2	L-3	L-4	L-5	L-6	L-11
C-1	24138	-	-	-	-	-	-
C-2	24379	16675	-	-	-	-	-
C-3	24708	17268	16053	-	-	-	-
C-4	25016	18005	16256	15333	-	-	
C-5	25314	18745	16638	15264	14484	-	-
C-6	25629	195844	17163	15402	14180	13481	-
C-11	26652	22059	19106	16728	14769	13148	8884

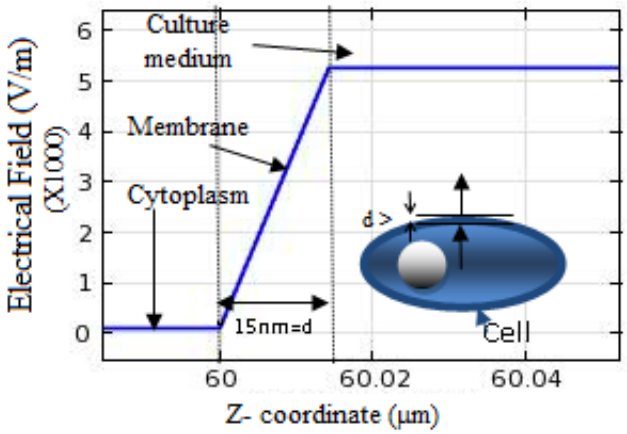

Figure 5. The EF dropped on the first layer of cells from cytoplasm to membrane and to medium. The transition of dropped EF on the cell membrane with a few nanometer thickness can also be seen.

B. Preliminary experimental results

Figure 6 shows the cultured fibroblast cells above gold IEs. In these figures, two gold electrodes (black) and cells can be seen. It is obvious a monolayer of cells is cultured where two electrodes are connected to ground and pulse generator. As expected the cells close to the edges are exposed to high electrical field intensity and therefore those are electroporated with PI. The phase contrast (b, c) and UV (d, e) microscopic images in two different electrical conditions are demonstrated in Figs. 6a-6d.The transfected PI molecules result in white color (Due to white and black imaging) of nucleus. Higher voltages applied on electrodes results in higher EFs. The generated bubbles in these figures are due to higher electrical field around the electrodes. Higher EF results in the release of hydrogen gas near the gold electrodes.

IV. DISCUSSIONS

Based on simulation and experimental results, the planar IEs generate non-uniform EFs resulting in non-homogenous transfection. We observed in experimental (Fig. 6) and simulation results (Fig. 3) that in a non-uniform EF, some living cells die (due to high EF while other cells even don't

transfected (due to low EF). Therefore, the planar IEs with large distance in between may decrease the performance of EP system (low viability and low transfection efficiency).

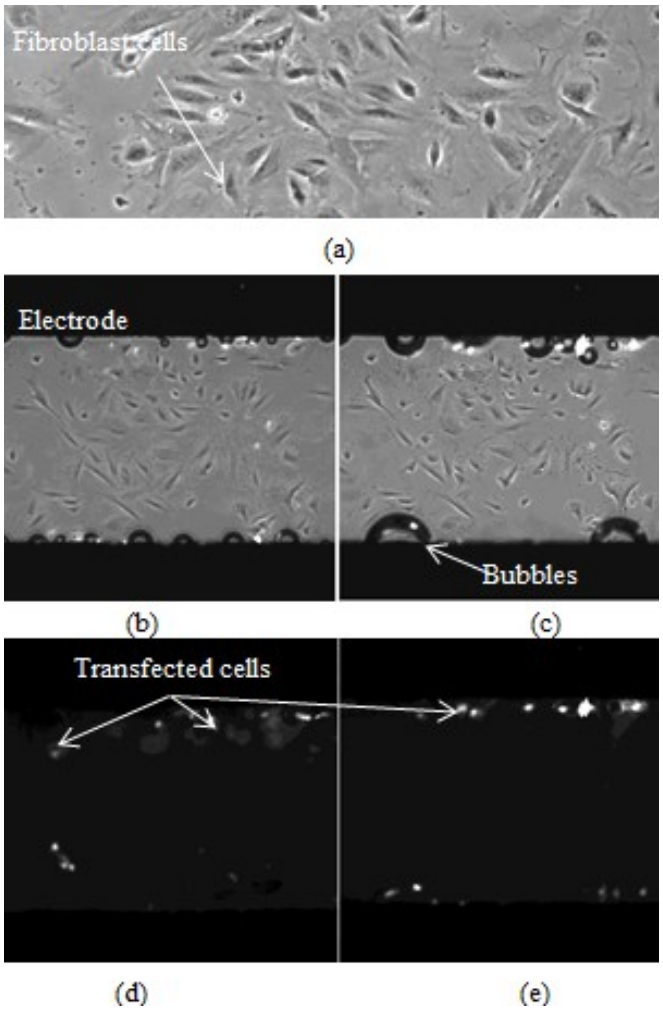

Figure 6. Microscopic images of (a) cultured fibroblast cells above gold electrodes, phase images of fibroblasts after the application of pulse voltage (b) 1 volt, 10 ms, (c) 1volt, 100 ms respectively and their corresponding UV microscopic images (d), (e).

In order to generate uniform electrical fields there are two techniques which are compatible with microelectronic chip technologies (Fig. 7): "Development of IEs with very small width and distance between the electrodes" and "Development of IEs with the second common ground electrodes above the cells culture medium".

The first method offers the advantage of low voltage and no need to another off-chip electrode; however, the fabrication of such nano-electrodes is not trivial. Another disadvantage of nano-scale IEs is that the depth of influencing EF is in the range of a few nanometers. Therefore, the topside of cells might not be electroporated and not transfected. The second method can simply be realized and offer the advantage of uniform EF throughout the medium. However according to the distance between the parallel electrodes, it may require higher voltage. This is why for cellular analysis, the standard CMOS process with higher voltages are employed to develop the microelectronic interface. Further post-processing should also be performed to coat the electrode with gold and other bio-friendly materials suitable for electroporation purpose. A microfluidic structure can also be designed to load the culture medium and cells in small microfluidic chambers bonded on MEs using Oxygen plasma technique.

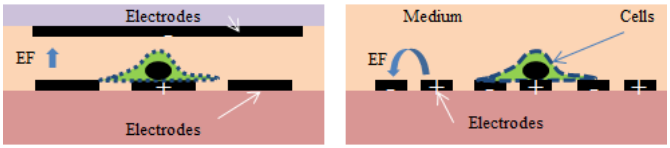

Figure 7. ME Configuration techniques: (a) Parallel and (b) Nano-scale IE.

V. Conclusions

In this paper, we discussed a transfection method using MEs which can be realized on standard microelectronic technologies. The roles of geometry and dimension of MEs on the cell viability and transfection efficiency were studied using two computational and experimental techniques which were in agreement. Based on these studies, as the future efforts, we will design the EP system on microelectronic chip for high throughput transfection of single cells suitable for reprogramming purposes.

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