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CARBON CONE ELECTRODES FOR SELECTION, MANIPULATION AND LYSIS OF SINGLE CELLS

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ABSTRACT

Here we present initial experiments towards an integrated platform for single cell selection, manipulation and lysis. The premise is that an array of polarized conical carbon electrodes can be dipped in a cell culture, trap cells of interest using dielectrophoresis and transport them to specific locations where they can be lyzed electrically. We aim at developing an automated tool to extract intracellular components from targeted particles over specific locations, *i.e.*, a DNA microarray or other functionalized spots. What we contribute in this work is modeling of the electric field and its gradient around carbon cones, as well as initial cone fabrication results. To the best of our knowledge, both the fabrication of conical glassy carbon electrodes and the general concept of the proposed platform are novel. Ongoing work is on demonstrating cell trapping and lysis using these conical electrodes by only varying the magnitude and frequency of their polarizing AC signal.

INTRODUCTION

Positioning single cells is of utmost importance in several areas of biomedical research: in vitro fertilization, cell-cell interaction, cell adhesion, embryology, microbiology, stem cell research, and single cell transfection [1]. Single-cell manipulations, using a micropipette, have been demonstrated to be reliable, minimally traumatic, and widely accepted. However, in the absence of cell selection and placement automation, it is time consuming, labor intensive, and unsuitable for processing large numbers of single cells [2]. There is still a great need for further improvement in miniaturization, integration and detection sensitivity. There is also a great need for automation; throughput and bioinformatics to study multiple individual cells to achieve statistical significance [3].Microfluidic platforms hold the promise of revealing the differences among individual cells that compose a group. Currently there is a barrier between the two approaches in the field; namely, a simple chip design that leads to a high throughput with limited details, and a complex design means many more details but low throughput. If a large number of cells can be individually addressed in a relatively simple microchip, both the throughput and quality of single cell data will improve. [5]

Our device integrates and automates the crucial steps of single cell capture, single cell lysis and deposition of the lysed cell material at specific sites selected for individual cells. The selective cell capture of single cells is enabled by the use of dielectrophoretic force applied between electrodes with a specific geometry. The array of electrodes will capture the cells

and transport them to designated sites for cell lysis. When the array is aligned with these sites, by changing the frequency of the electric field employed, the cells will be lysed and the intracellular material will be deposited at the sites. Here we present initial results towards this system.

THEORY

Three main parameters are of our interest are the selectivity and strength of a trapping force, the drag force on the trapped particle during translation and the electric field required for lysis. We seek an electrode shape that offers precise volumes of specific trapping force strength to keep a single particle in place during movement and yields strong enough electric fields for lysing only when necessary, *i.e.* once the cell is on top of a specific site location. It has to be ensured that, when the cell is selectively captured, the electric field is not high enough to lyse the cell. Here, we have done the analysis for selective trapping and lysis of the yeast cells, assumed to be spherical with the radius of 2-4 μm.

The use of dielectrophoretic force to capture cells is advocated here. Dielectrophoresis (DEP) is the lateral movement of particles induced by polarization effects in non-uniform electric fields (Pohl, 1978; Pethig, 1979; Jones, 1995). The DEP force on a particle depends on its dielectric properties relative to that of the external medium, as well as on the frequency and degree of non-uniformity of the applied electric field. This can enable selective trapping of the particle. [7] The dielectrophoretic force F_{DEP} acting on a circular particle depends on the gradient of square of electric field (∇E^2) according to the equation [5] :

$$F_{DEP} = 2 * \Pi * r^3 * \varepsilon_m * Re[K(\omega)] * \nabla E^2 \quad (1)$$

Where, r is radius of the particle, $\varepsilon_m$is permittivity of the medium, $Re[K(\omega)]$ is the real part of the Clausius Moseti factor. Thus, the calculation of the electric gradient is an important factor for determining the force of cell capture.

Once the single cell is captured at the tip of each electrode, the array is transported through the medium to the deposition sites. The Hydrodynamic drag force acts on the captured cells during this transport opposing the dielectrophoretic force acting on the cells. The hydrodynamic drag force F_{Drag} on a spherical particle is related to the flow velocity by the Stokes equation:

$$F_{Drag} = -6 * \pi * r * \nu * \eta \quad (2)$$

Thus, to ensure cell attachment to the electrodes during transport,

$$F_{DEP} \geq -F_{Drag} \quad (3)$$

Where η is the viscosity of the fluid r is the radius of the particle and v is the flow velocity.

When a cell is exposed to an external electric field, the transmembrane potential Vmem is induced. If the transmembrane potential is higher than about 1 V, the membrane is permeable to an outside medium. When the electric field produces a transmembrane potential higher that 1 V, as shown in Fig. 2, the membrane is disrupted irreversibly. [3] Thus, during capture, it is desired that the cell membrane potential does not exceed 1V. The cell membrane potential for a spherical particle is calculated using the equation [4]:

$$V_m = \frac{1.5 * E * r \cos\alpha}{\sqrt{1+(2\pi f \tau)^2}} \quad (4)$$

Where, $\tau = r * C_{mem} * (\frac{1}{\sigma_{cyto}} + \frac{1}{2*\sigma_{medium}})$

V_m is the cell membrane potential, E is the applied electric field, r is the radius of particle,. α is the angle between the field line and a normal from the center of the particle to a point of interest on the membrane. In this work α equals 0° to account for maximal trans-membrane potential, σ_{cyto} is the conductivity of cytoplasm σ_{medium} is the conductivity of the suspending medium and C_{mem} is the capacitance of the cell membrane.

METHODOLOGY

The initial phase of the work was modeling the channel for cell suspension and the electrode array for cell capture and simulate the electric field and electric field gradient. These simulations were carried out on COMSOL Multiphysics 4.4 (COMSOL Inc,Sweden) on the Windows 7 Enterprise 64-bit operating system with 32GB RAM and Intel® Xeon ® CPU E5-1650 v2 @ 3.50 GHz processor. Geometry of an electrode dictates the distribution of electric field and gradient around the electrode. In this work, we studied electrodes of a shape of a cone with curved tips for their characterization, fabrication and possible use. This shape was selected as it gives an advantage of narrow tip, where the electric field and gradient is high. Conical electrodes are characterized by the base radius, the apex radius, and the angle made by the axis with the surface. The geometric model constructed in COMSOL is shown in Fig.1.Paramters considered are given in Table.1.

A small block was defined around an electrode calculate the volume of the electric gradient around the electrode tip. This particular electrode was chosen to represent an electrode in the array surrounded by six other electrodes and thus influenced by their potentials. The domain was meshed using fine tetrahedral meshing for the entire region for 5 iterations with the maximum element depth of 8 microns. The inner domain consisting of the electrodes was refined for two further iterations using the method of splitting the largest side. The number of mesh elements was about 90,000. The fig.2 shows meshing of the domain. Water (a built in material in COMSOL 4.4) was selected as the material for the channel with the relative permittivity value equal to 80.3 and the conductivity value as in table 1. The electrode material was defined to be carbon with resistivity of the material equal to

$1*10^{-4}$ Ω.m. Physics defined for the model was 'Electric Currents'. Two walls were set as floating boundaries to simulate the inlet and outlet of the channel. All other walls of the domain were set as electric insulators by default. A stationary study for BICGStab iteration solver was used to compute the values of electric field and electric gradient. Factor of error estimate was 400 and the maximum number of iterations run was 10000.

Serial Number	Parameter	Value
1.	Base Radius of cone	10,20,25 µm
2.	Semi angle of cone	Angles 12˚to 60˚ (increment 6˚)
3.	Tip Radius of cone	0.5,1,2 µm
4.	Voltage applied between cones	2,5,10 and20 V
5.	Distance between Cones	85µm
6.	Conductivity of Medium	0.001S/m

Table 1. Parameters for Simulation of the domain

The equations solved were:

$$\nabla . J = Q_j \quad (5)$$

$$Q_j = 0 \ (in\ this\ case\ due\ to\ absence\ of\ external\ charge$$

$$J = \sigma E \quad (6)$$

$$E = -\nabla V \quad (7)$$

Where Q_j is the electric charge in the domain, J is the charge density, E is the electric field, V is the voltage and σ is electrical conductivity of the medium.

The gradient of square of electric field (∇E^2) was defined by mathematical calculation of gradient as:

$$E = Ex\hat{\imath} + Ey\hat{\imath} + Ez\hat{\imath} \quad (8)$$

$$E^2 = Ex^2 + Ey^2 + Ez^2 \quad (9)$$

Thus, the gradient of square of electric field(∇E^2), can be computed as:

$$\nabla E^2 = \left(\hat{\imath}\frac{d}{dx} + \hat{\jmath}\frac{d}{dy} + \hat{k}\frac{d}{dz}\right) * (Ex^2 + Ey^2 + Ez^2) \quad (10)$$

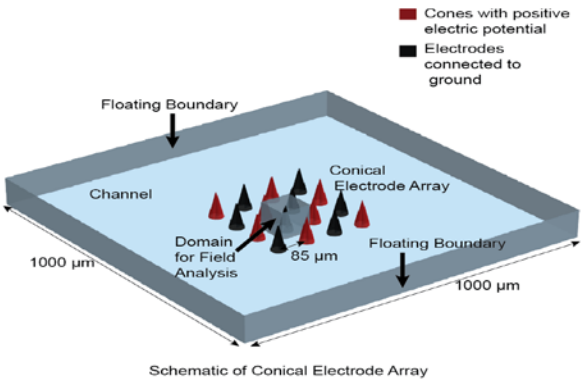


Fig.1 Geometry of the COMSOL Model

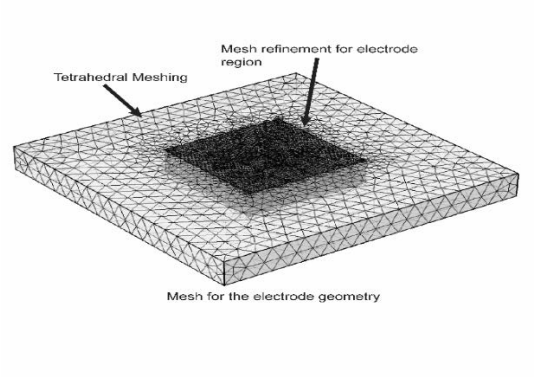
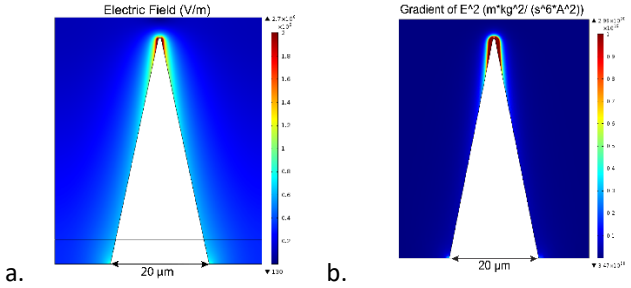


Fig.2 Meshing of the Domain

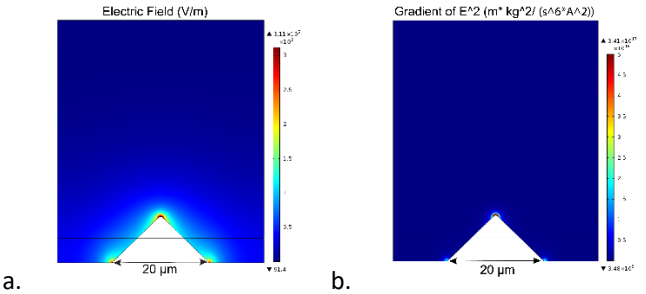
RESULTS AND DISCUSSION

The values of electric field and electric field gradient were obtained. Fig.3 and Fig.4 show the Electric field and gradient simulated for a cone with semi angle 12˚ and 45˚ respectively. It can be seen that the high gradient region is concentrated at the tips of the geometry. Also, the concentrated volume is higher at 12˚ than at 45˚.



a. b.

Fig.3 Electric Field (a) and Gradient (b) simulated for geometry with semi angle 12˚ and base radius 20 µm at 2V



a. b.

Fig.4 Electric Field (a) and Gradient (b) simulated for geometry with semi angle 45˚and base radius 20 µm at 2V

The values for the maximum electric field were obtained at each angle and by using equation 4, the maximum electric field at which cells can be captured without lysing for the three frequencies of electric current, 1e3, 1e4, and 1e5 Hz was calculated. These results are shown in Fig.5.It can be seen from Fig.5, that the values of electric field obtained at all angles other than 18˚ can successfully capture cells at the frequency of 1e5 Hz without lysis. Angles above 30˚ and the angle of 12˚can be effectively used for the frequency of 1e4 Hz.

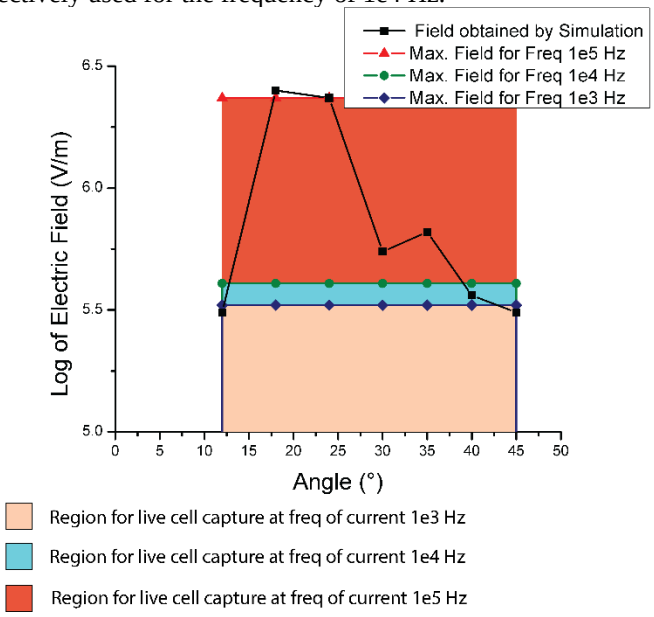


Fig.5 Plot of the electric field at various angles and the maximum field sustained at various semi angles of cone for 2 micron yeast cells without lysing at 2V

If the frequency of electric field selected is 1e3 Hz, none of the models except the angle 45˚ can capture cells effectively without lysing them. The maximum velocity of the transport for the array can be determined based on the gradient of the electric field for the different models and is shown in Fig.6. A single cell should be trapped at the tip of the conical electrodes. To find if such trapping was feasible, the volumes of the region with desired values of DEP force were calculated. This was done using the derived values obtained by interpolation function inbuilt in COMSOL. It was found that, the maximum value of the gradient occurred at the tip of the electrode and had a value of around 1e17-1e18 ($m.kg^2/S^6.A^2$). It was observed that the results for this gradient value were acceptable for the voltage of 2V and 5V for the given conductivity. These are plotted in Fig.7

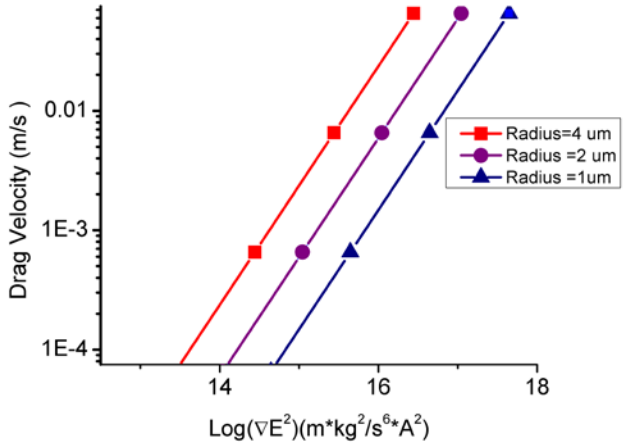


Fig.6 Plot of the drag velocity vs the log value of the ∇E^2 for yeast cells with radii 1, 2 and 4 µm.

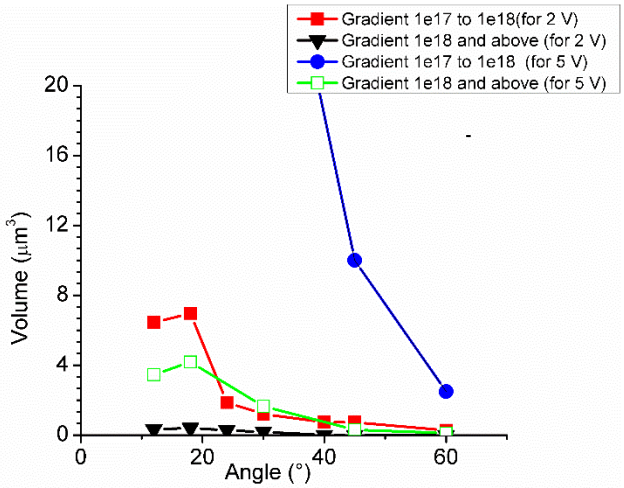


Fig.7 Plot of the Volume of highest gradient for different angles at the tip.

Different plots (Fig.8 and 9) to demonstrate the points satisfying all the three parameters were further obtained for the voltage of 2V and 5V. These figures enabled us to compare different parameters with the cone angle. Fig.9 shows the angles with a favorable capture volume and cell membrane potential. The same point can be studied on the Fig.8 to obtain the drag force and hence determine the velocity.

ONGOING WORK AND CONCLUSION

Current results demonstrate that the simulations give favorable results for single cell capture at the electrode tips. The frequency of the electric field can be initially set to be 1e5 or 1e4 Hz depending on the geometry chosen. When the electric field is

applied at this frequency, the cells can be successfully captured without lysis. When the panel of lysed cells is moved to the section for cell lysis and material deposition, the frequency of the electric field will be decreased below the threshold value and the cells will be lysed. The placement of the panel with electrodes exactly above the sections for cell capture can facilitate the deposition of the cell material at desired locations. Current work includes simulation by considering different media and analyzing the effect of media conductivity on the cell capture. The study is also being extended to different cells.

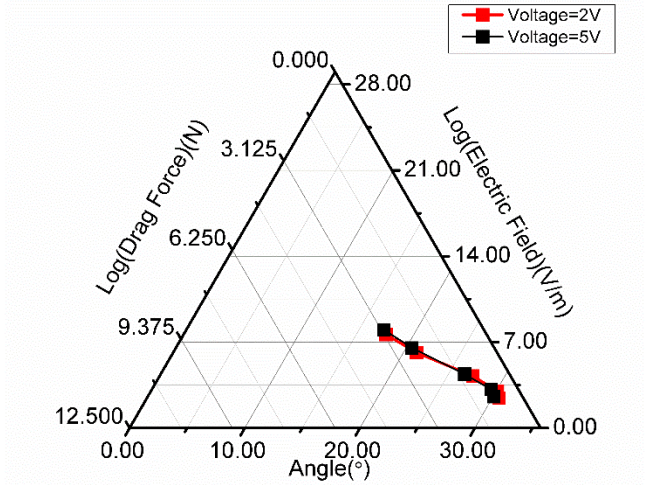


Fig.8 Plot of the drag force and Electric field for various geometries.

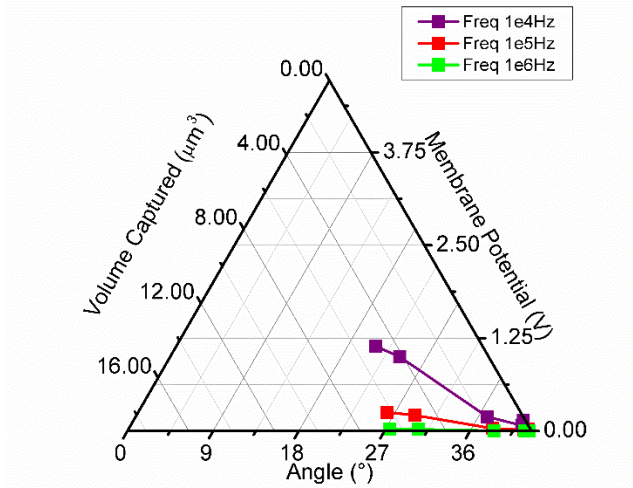


Fig.9 Plot for the volume captured and cell membrane potential for various geometries

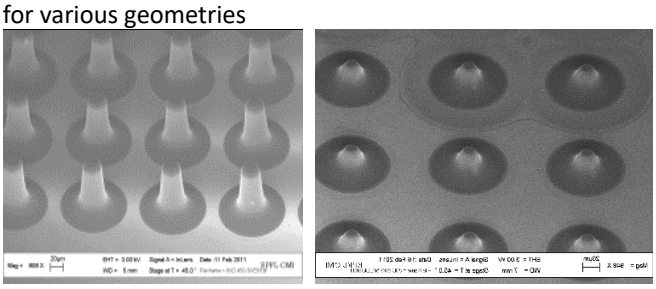


Fig 610. Carbon cones obtained by photolithographic process

Fabrication of electrodes of the desired geometries is also in progress. Glass-like carbon is the desired material for fabrication of the electrodes. Glass-like carbon has a wider electrochemical stability window than platinum and gold, which makes it ideal in electrochemistry experiments. [6] We are using carbon electrodes obtained by photolithography followed by pyrolysis for this process. The different shapes obtained using these electrodes are shown in Fig.10.The process starts with two step photolithography of a carbon precursor on a transparent substrate such as fused silica and quartz. Use of transparent substrate allows back exposure which is necessary for the fabrication of cones. SU-8 is used as the precursor to carbon. The photolithography process comprises of following steps: 1) photo-patterning of 10 µm thick layers of SU-8 by front exposure to fabricate the connecting leads to the cones; 2) photo-patterning of 200 µm thick layers of SU-8 by back exposure to fabricate the cones. The cones are fabricated on the connecting leads by proper alignment. Carbonization at 900 °C in an inert atmosphere turns the photo-patterned SU-8 structures into carbon cone electrodes. A heating ramp of 5-10 °C is generally implemented during carbonization. 95% carbon yield is expected in this heat treatment process. Ongoing task is to optimize these parameters to obtain above simulated geometries.

Automation of the device and testing will be performed as the future steps to validate the working of the device.

REFERENCES

[1] T. P. Hunt, R. M. Westervelt, Dielectrophoresis tweezers for single cell manipulation, Biomedical Microdevices, Volume 8, Issue 3, pp 227-230,2006

[2] Yasser H. Anis, Member, Mark R. Holl, Member, and Deirdre R. Meldrum, Fellow, Automated Selection and Placement of Single Cells Using Vision-Based Feedback Control, IEEE transactions on automation science and engineering, VOL. 7, NO. 3, 2010

[3] A continuous electrical cell lysis device using a low dc voltage for a cell transport and rupture, Dong Woo Lee, Young-Ho Cho, Sensors and Actuators B:Chemical, Volume 124, Issue 1, 10 June 2007, Pages 84–89

[4]Zimmermann, U. and Neil, G. A., Electromanipulation of Cells CRC Press, Boca Raton, FL, 1996.

[5] Microfluidic Platforms for Single-Cell Analysis
Annual Review of Biomedical Engineering
Vol. 12: 187-201, August 2010
Richard N. Zare and Samuel Kim

[6] Optimization of Carbon Electrodes Derived from Epoxy-based Photoresist, Andrea Mardegan, Rahul Kamath, Swati Sharma, Paolo Scopece, Paolo Ugo, and Marc Madouc, J. Electrochem. Soc. 2013, volume 160, issue 8, B132-B137

[7] Dielectrophoretic separation of bacteria gradient using a conductivity gradient, Gerard H. Markx, Penelope A. Dyda, Ronald Pethig, Journal of Biotechnology,1996.