Design of a Dielectrophoretic Based Micropipette for Gene Expression Applications Using COMSOL Multiphysics® Software

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Abstract

Introduction: Diagnosis are based on probing the critical genetic changes taking place in various stages of tumor development[1]. Currently, this is performed by assuming all the cells in the tumor are genetically identical[1]. However, current literature reports indicate that this assumption is incorrect[1,2]. To address this need, we have developed a technology that is capable of analyzing genetic changes in single-cells[2].
Our proposed technology is based on insertion of micropipette with fabricated electrodes into single-cells and extraction of mRNA (representative of genes) molecules through dielectrophoresis (DEP) (Figure 1). A positive DEP is generated on mRNA molecules by applying external electric potential and forcing them to move and towards the apex of the micropipette. Further, we have attached capture probes (single-stranded DNA molecule complementary to the target mRNA) on the apex of the micropipette. The target mRNA molecules hybridize with capture probes. After hybridization, the pipette is withdrawn out of the cell, and mRNA molecules are collected into a micro-well applying a negative DEP. Finally, extracted molecules are quantified using traditional RT-PCR techniques.
Use of COMSOL Multiphysics®: We have used COMSOL Multiphysics® to understand the electric fields and electric field gradients around the micropipette. In particular, two-dimensional axis-symmetric model was developed using the electric circuit physics under AC/DC Module. An AC voltage (1Vpp, 120 kHz) was applied between electrodes, and a frequency domain analysis was performed at 120 kHz. Using a user defined dense mesh, first, the variation of electric potential was calculated. However, we were interested on the magnitude of $\nabla(E^2)$ because the DEP force is directly proportional to $\nabla(E^2)$[3]. Therefore, using the potential, we have also calculated the variation of E and $\nabla(E^2)$ in the vicinity of the micropipette assuming micropipette was placed inside a cell (Figure 2). It is necessary to optimize the apex diameter to generate high DEP force on mRNA molecules. Therefore, series of simulations were performed and calculated variation of $\nabla(E^2)$ and E with apex diameter (Figure 3). In addition, using these results, we also calculated the cell volume in which the DEP is large enough to capture mRNA molecules. This is called as the capture volume and it is defined as the region where $1/2\ \alpha E^2 \geq kT$ is satisfied, where k is Boltzman constant and T is absolute temperature, and α is polarizability of mRNA molecules[4]. Figure 4 illustrates the calculated capture volume.
Results: Our COMSOL model analysis indicates a large DEP on mRNA molecules. To the best

of our knowledge, this is the largest ∇(E^2) ever reported and it is very important to generate a large ∇(E^2) to have a high-throughput gene expression profiling. The calculated capture volume is about 20% of cell volume. As millions of mRNA molecular copies present in the cell, this capture volume would be sufficient to effectively extract mRNA molecules from this cell biopsy.
Conclusion: We have successfully utilized AC/DC module to calculate∇(E^2) and E. We have also calculated the capture volume of the micropipette. Currently, experiments are performed to find other important parameters such as range, throughput and sensitivity.

Reference

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Figures used in the abstract

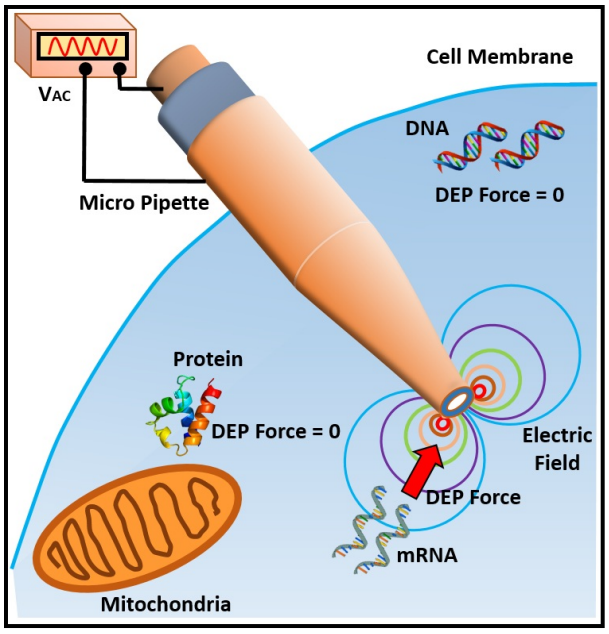

Figure 1: Schematic representation of the DEP based single-cell mRNA extraction technique.

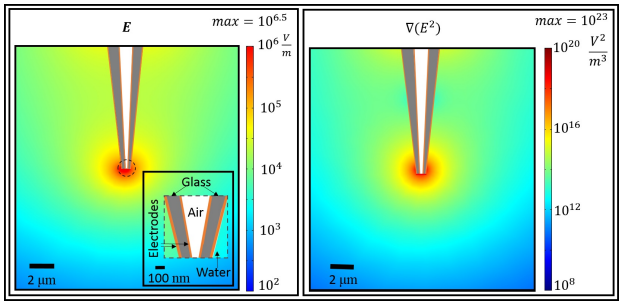

Figure 2: Variation of E and ∇(E^2) in the vicinity of the micropipette assuming micropipette was placed inside a cell.

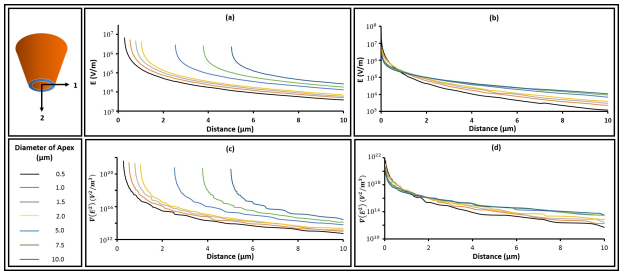

Figure 3: Variation of E and ∇(E^2) with apex diameters. (a) Variation of E along horizontal direction (line 1). (b) Variation of E along vertical direction (line 2). (c) Variation of ∇(E^2) along horizontal direction (line 1). (d) Variation of ∇(E^2) along vertical direction (line 2).

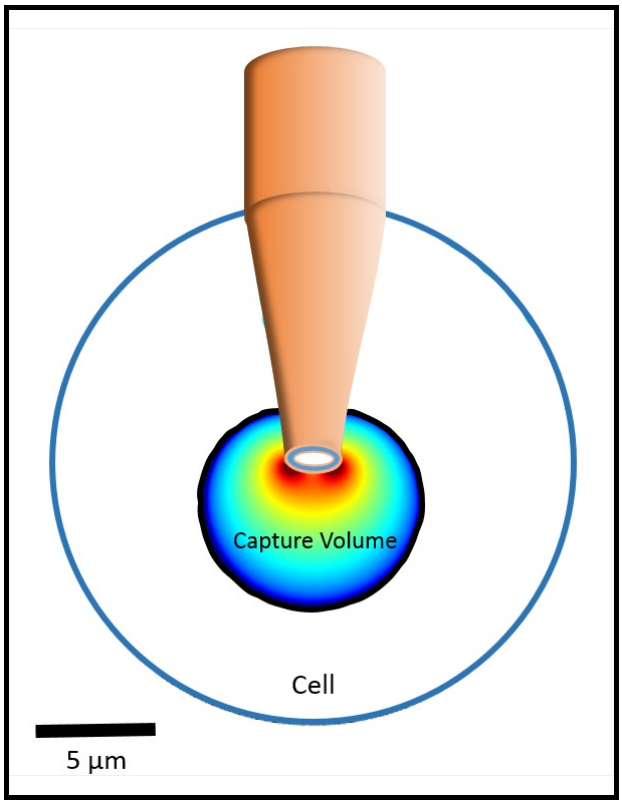

Figure 4: Calculated cell volume (in rainbow color) where micropipette extract mRNA molecules using DEP.