Single-phase dielectrophoretic and electrorotation studies using three dimensional electrodes for cell characterization

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***Abstract-* A novel electrokinetic approach using single-phase electrorotation for the label-free manipulation and characterization of biological cells is presented. A single shell model was used to theoretically design and develop an experimental strategy for biological particle characterization. As a study model, electro-rotation of glutathione agarose (GA) beads was studied using three- dimensional spatially oriented micro-needle setup. Effect of electrical parameters (i) voltage: from 0 – 10 Vpp and (ii) frequency: 0 -100 MHz was evaluated on a heterogeneous mixture of GA beads (35 -150 microns). The relationship of the electrical parameters to rotational frequency of the beads was studied. This paper demonstrates a simple and easy to implement prototype for electrokinetic characterization of particles with translational potential for biological cells.**

I. INTRODUCTION

Cell manipulation and characterization is critical for biomedical research that involves study of cell-cell interaction, cell adhesion, *in-vitro* drug effects on cells, microbiology, embryology, and regenerative medicine[1]. Understanding the function of cells and their interaction within a micro-environment will reveal information about cell morphology and bio-chemical communication inside/outside the cells[2]. Manipulation of cells has been achieved through techniques which focus on physical parameters as well as biochemical affinity. Such techniques include micro-centrifugation, optical tweezers, antibody-affinity cellular studies, fluorescent activated cell sorting (FACS) and other microfluidic technologies[8]. However, commercial applications using the above techniques have distinct advantages and disadvantages with respect to efficiency of cell manipulation, throughput of the system, purity and recovery of the cells under study[2].

As the demands placed on cell characterization in the field of medicine and research continues to increase, novel solutions to overcome the identified limitations are being designed and implemented[5]. One such novel technique is the development of electrokinetic phenomena such as dielectrophoresis and electrorotation, which are used to study cells under the influence of applied electric fields in a label-free manner[3]. Using standard electrokinetic methods, inherent passive dielectric properties can be determined to understand the electrical communication of the cells[4]. However isolation and characterization of specific cell subtypes from a heterogeneous cell population through standard techniques faces significant challenges[1]. They also require sophisticated planar electrode setup and complex fabrication procedures. In this paper, we demonstrate the design and application of single-phase electrorotation using a simple to fabricate device and reconfigurable three-dimensional micro-needle electrodes. The particles under study are analyzed to understand their unique response to the applied single-phase uniform electric fields.

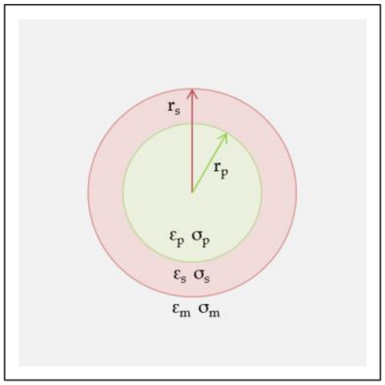

Figure 1: The Single-shell model represented by a spherical particle with radius r_p, permittivity ε_p and conductivity σ_p enclosed by a single shell of radius r_s, permittivity ε_s and conductivity σ_s suspended in a medium of permittivity ε_m and conductivity σ_m

II. MATERIALS AND METHODS

A. *Theory*

Non-polar particles in aqueous solution will undergo polarization when exposed to an applied electric field. The interaction between electric field and dipoles formed as a result of polarization generates a lateral force. The dielectrophoretic translational force that is produced is inhomogeneous due to the presence of gradient electric fields. This causes the particle to move towards high or low electric field regions[7]. The movement is largely dependent on the voltage applied, frequency of the signal and dielectric properties such as conductivity and permittivity of the particle and medium. Time averaged dielectrophoretic force (as shown in Eq. (1)) exerted on a particle of radius R is given by

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

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where K (ω) is the frequency dependent Claussius-Mossotti factor that determines polarizability of the suspended media, expressed by Eq. (2); ∇E^2 is the gradient electric field; ε_m is the permittivity of the medium; and ε_p is the permittivity of the suspended particle. ε_m^* and ε_p^* are the complex permittivities of both particle and media that can be expressed by Eq. (3). The permittivity of a medium/particle determines the rate and efficiency of its polarizability[1].

$$K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \quad (2)$$

$$\varepsilon^* = \varepsilon - \frac{j\sigma}{\omega} \quad (3)$$

Several studies have shown various applications such as cell separation and fractionation based on the attractive and the repulsive forces experienced by the cells. The real part of the CM factor determines the force experienced by the particle and the direction of motion. However, the imaginary part of the CM factor aids in understanding the self-induced rotational force, also known as electrorotation torque, experienced on the particle when subjected to uniform gradient electric fields[6]. The torque of the particle in a three-dimensional rotating single-phase electric field is given by

$$\tau = -4\pi\varepsilon_m r^3 Im[K(\omega)]E^2 \quad (4)$$

The imaginary part of the torque is determined by the dielectric properties of the microenvironment, which can be tuned by three dimensional spatially oriented phase electric fields. Also, the frequency dependent Re [K(ω)] and Im[K(ω)] indicates the permittivities of the solution and particle as a function of frequency. From the equation (1) & (4) it is evident that the magnitude of the dielectrophoretic force and rotational torque is significantly affected by the particle size (R) while other parameters such as gradient electric field, voltage applied and frequency can be controlled. Changes in the behavior of particle-field interactions can be observed as a result of reduction in size due to variation in generated force.

In order to completely understand the particle behavior and its interaction with the applied electric field, inherent dielectric properties of the particle plays a major role. The common approach to estimate the design parameters to identify the unknown inherent properties of the particle is to theoretically model the particle through multi-shell model[1]. For this study, we considered a generalized multi-shell cell model with only one shell, single-shell spherical particle. Study model is considered to be functional with high internal conductivities. The effective dielectric properties such as conductivity and permittivity for approximation are represented using figure 1. The complexity beyond single shell model involves multiple permittivity values and conductivities for various layers of the particle in study. For this study, the effective polarizability and the dipole moment can be represented as the following equations.

$$\tilde{\alpha} = 3\varepsilon_1\widetilde{f_{CM}} = 3\varepsilon_m\left(\frac{\widetilde{\varepsilon_{23}} - \widetilde{\varepsilon_1}}{\widetilde{\varepsilon_{23}} - 2\widetilde{\varepsilon_1}}\right) \quad (5)$$

$$p = 4\pi\varepsilon_1\widetilde{f_{cm}}a_1^3 E \quad (6)$$

In this study, we demonstrated the influence of uniform electric fields on synthetic agarose beads. The beads were attracted or repelled at optimal single-phase frequencies and voltage values. Also the particles could self-induce rotation under the application of single-phase electric fields. The particles were able to exhibit both translational and rotational motion under the influence of uniform single-phase gradient electric fields.

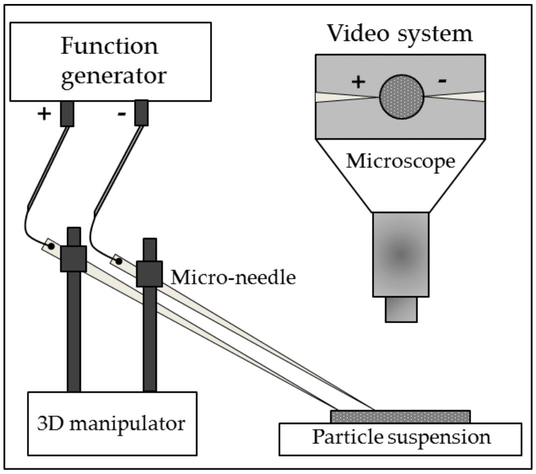

Figure 2: Two three-dimensional micro-needle electrode setup for particle characterization using single-phase electrokinetic techniques

B. *Fabrication of single-phase electrorotation device*

To test our study model, two micro-needle electrode setup was used as shown in figure(2).The three-dimensional spatially oriented micro-electrodes used for this study are gold coated fine (30 gauge) dermal needles with an approximate diameter of 100µm (Fisher Scientific, MA). The two needles are separated at a center to center distance of 500 µm for maximum electric field confinement and observe size matching to the particle of interest. 200nm of gold metal was cryo-evaporated on to the needles and it was chosen as it is biologically inert and offers good conductivity. A 50 nm chrome layer was used as the base adhesion layer for gold metal. The needles were then housed on a liquid encapsulating chamber fabricated with polydimethoxysilane (PDMS) (Dow Corning,MI). The setup was plasma bonded to a microscope glass slide for visualization under an optical microscope (Leica Microsystems,IL). After housing the needles, 0.2mm diameter aluminum connector wires were placed in electrical contact with the micro-needles using temperature cured silver conductive epoxy. Zero phase–shifted AC sinusoidal voltage varying from 100mVpp to high voltage of 10Vpp was applied to produce uniform gradient electric fields. Frequency of the applied sinusoidal voltage was also varied from 100mHz to a very high frequency of 100MHz.

C. *Sample preparation*

As a study model, spherical glutathione agarose (GA) beads were chosen (Sigma Aldrich, MO). The beads are biocompatible and their characteristics depict the simple single-shell model. GA beads were surface functionalized using Glutathione S-Transferase (GST), glutathione peroxidase and glyoxalase 1 along with 4% cross-linked beaded agarose making it highly biocompatible and cell like study model. Also, the buffer media was diluted with 20% ethyl alcohol to prevent the beads from disintegrating and 1% BSA was added to adjust the conductivity of the media.

BSA also prevents the beads from sticking to the glass substrate or to the micro-needles. The stock concentration was diluted 1000X solution with the buffer media to reduce the number of particles in suspension so as to clearly observe the electrokinetic effects on the target particle. Low conductivity was maintained throughout the experiment to reduce joule heating effects caused by the application of alternating current electric fields. A conductivity meter (Fisher Scientific, MA) was used to measure the conductivity of the media used. This was maintained constant at 50μSm^{-1}.

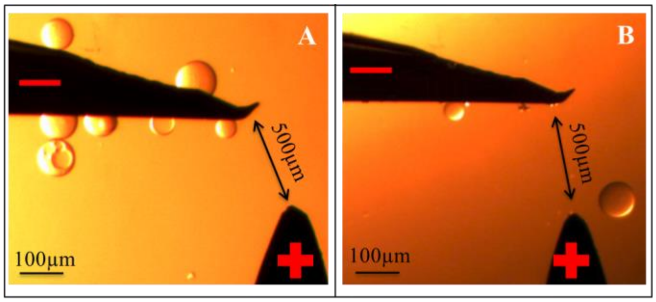

Figure 3: Capture images indicates the attraction (A) and repulsion (B) action of glutathione particles towards and away from three-dimensional two micro-needles. The overall time taken for these capture frames is < 0.1 second to observe particle motion.

III. RESULTS AND DISCUSSION

A. *Dielectrophoretic manipulation of particles*

Dielectrophoretic manipulation of glutathione agarose beads were performed using the micro-needle device setup using stationary fluid entrapment, for better understanding the effects of single-phase electric fields on suspended model beads. Conductivity measurements of the sample solution with GA beads were performed prior to the experiment and was determined to be approximately between 0.24 and 0.54 S/m. Prior to application of electric signal the suspended beads in PDMS well was left undisturbed for 15 minutes to create equilibrium within the test system by reducing the random motion of beads. This allowed for the understanding of the effects of electric fields on the stable system thereby eliminating the need for noise reduction within the system. Consequently, zero phase-shifted AC sinusoidal voltage ranging from 100 mVpp to 10 Vpp was applied with varying frequencies from 100 mHz to 100 MHz between the two micro-needles via a function generator. When the AC voltage was applied, the heterogeneous mixture with varying sizes of glutathione agarose beads was polarized. At 4 Vpp voltage and 100 Hz frequency, attraction of beads to the micro-needles was observed as a result of experienced dielectrophoretic force. The attraction of beads towards the micro-needles provides information about the polarizability of the particles. When the polarizability of the particle is higher than that of the medium, the particles move towards the field generating micro-needles and vice-versa. When the electrical parameters from equation (1) were changed to 10 Vpp voltage and at extremely high frequencies, repulsive forces were experienced by the particles as seen in figure 3 (a & b).

B. *Single-phase electrorotation measurements*

Cell rotation was captured using a CCD camera with a fixed frame rate of 20/s. the image captured was performed with a focus on the target particle. Post-processing image analysis algorithm that was developed identifies feature points on the particle from one capture image to another resulting in the determination of rotational frequency of the particle. The single-phase electrorotation of particles was observed when the micro-needles were charged with a voltage of 5.5 Vpp at 4 MHz frequency. Influence of the electrical parameters on the rotational frequency of the particle was observed over a spectrum of frequencies from 100 mHz to 100 MHz and a spectrum of voltages ranging from 100 mVpp to 10 Vpp. Series of captured images showing rotation of particle based on feature points is shown in figure 4.

C. *Rotational frequency vs. applied frequency*

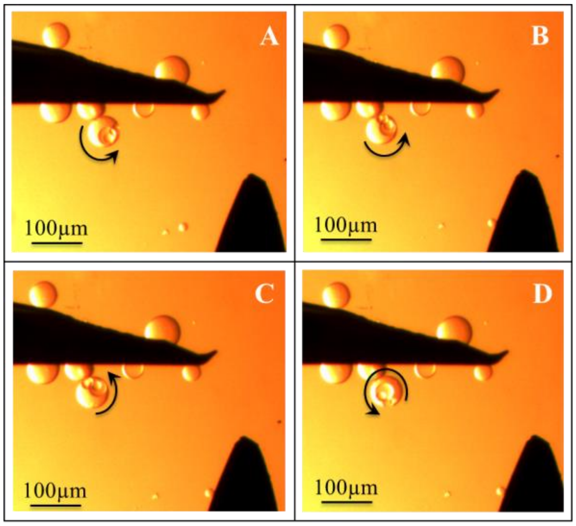

Figure 4: Capture images (A – D) indicates the rotational path of ~90 μm target glutathione particle using three-dimensional two micro-needle setup. The overall time taken for these capture frames is ~ 0.5 seconds.

Figure (5) shows the rotational frequency of glutathione agarose beads at applied frequencies from 100 mHz to 100 MHz. The GA beads of approximately 90μm were targeted to rotate under the application of the single-phase electric fields. The cells were originally at rest when no field was applied. By increasing the applied frequency from 100 mHz, the particles were attracted towards the micro-needles. They started self-induced rotation at constant phase gradient fields at 15 rpm at 4 MHz when a constant voltage of 10 Vpp was applied. The rotational frequency increased to 24 rpm at 6 MHz and slowly decreased to 15 rpm at 7 MHz. At much higher frequencies the particles experienced repulsive forces hence moved away from the micro-needles and eventually stopped rotating.

D. *Rotational frequency vs. applied voltage*

Figure (5) shows the rotational frequency of GA beads at applied voltage (Vpp). Particle size of approximately 90μm was focused to observe for self-induced rotation. The beads were originally at rest under the application of 0–2 Vpp.

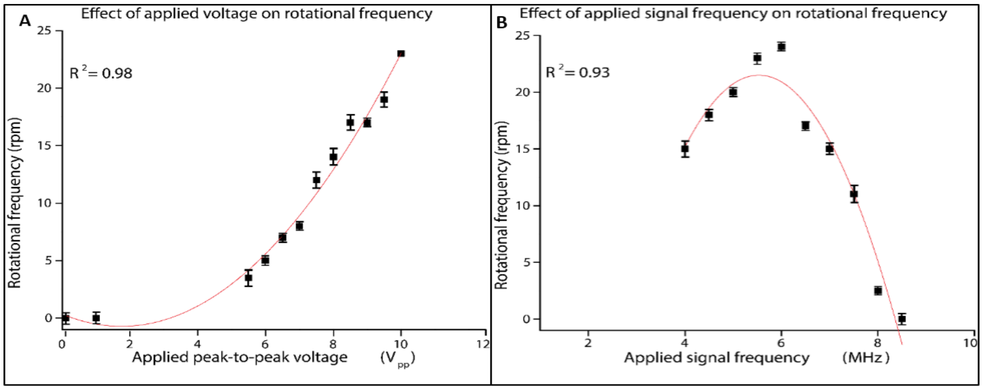

Figure 5: A) represents the effect of applied signal frequency on rotation frequency of glutathione agarose beads over a signal frequency spectrum of 0 to 10 MHz and B) indicates the relationship between applied signal peak-to-peak voltage on rotational frequency. A voltage sweep of 0 – 10 V_{pp} was performed. Error bars represent standard deviation over mean calculated for n=5 replicates.

The beads experienced attractive forces towards the micro-needles and eventually started to rotate at 3.5 rpm at 5.5 Vpp. The rotational frequency increased with an increase in applied electric field thereby creating uniform gradient electric fields. At 10 Vpp the rotation frequency reached maximum at a frequency of 23 rpm at 6 MHz frequency. The plot indicated a quadratic relationship between the rotational frequency and the applied voltage. It is obtained by fitting a polynomial curve to understand the response of the particle with respect to the signal voltage. During the application involving cells, as the cells are made up of bilayer and are conductive in nature upon polarization, rotation can be observed at much lower voltages, approximately less than 5Vpp.

IV. CONCLUSION

These results show the ability to manipulate and characterize beads using three dimensional electrodes and single-phase alternating voltages. One can separate different sized beads or beads of different composition based on the application of specific electrical parameters of the signal resulting in unique lateral/rotational forces. The simple to fabricate setup was tested for unique characterization of GA beads at voltages ranging from 5.5 Vpp to 10 Vpp over a frequency spectrum varying from 4 MHz to 7 MHz at a stationary fluid setup, to reduce external noise due to fluid drag. Overall n=5 replicates were performed to determine the efficacy of the model system. The observed results demonstrate the ability to self-induce rotation in particles using zero phase-shifted electric signal. After performing initial set of experiments, higher concentrations of model beads were used to study the effectiveness of the system. This single-phase electrokinetic method can be used to analyze and identify the unknown dielectric properties of the particles in suspension. Much experimental work remains to be performed to derive a complete prototype model for single-phase electrorotation. However, these experimental results will serve as guiding factors for design strategies of a novel electrokinetic cell characterization tool. This can further be applied in characterization of isolated biological particles where specificity of target cells is critical. For example, differences between cancer cells and cancer stem cells can be well characterized without probing them with any biomarkers that might affect the cellular properties. Also, rare cells can be studied and analyzed for their dielectric and physical characteristics without compromising on the purity and recovery of the target cells post-analysis.

V. REFERENCES

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