DIELECTROPHORESIS-BASED 3D CELL ROTATION THROUGH INTEGRATION OF BOTTOM AND VERTICAL ELECTRODES

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ABSTRACT

Dielectrophoresis rotation of particles in 3D has important applications in biological cell manipulation, sorting and characterization. This paper reports the rotation of spherical bovine oocytes in yaw and pitch axes and their angular velocity spectrum by generating dielectric torque on the oocytes inside the chamber of a fabricated biochip. We predict by numerical computation and confirm experimentally that bovine oocytes are permanently polarizable with no Maxwell Wagner interfacial relaxation at critical frequency. The capability of rotating particles in 3D opens new research opportunities in cell manipulation and analysis.

KEYWORDS: Dielectrophoresis, Electrokinetics, Enucleation, 3D rotation

INTRODUCTION

Precise translation and rotation of biological entities, such as cells, is a fundamental manipulation requirement in applied biotechnological research. Unlike translational manipulation, rotation remains extremely challenging when it is simultaneously required for more than a single axis for cell orientation in three dimensional (3D) spaces. Two typical case applications are cell injection and enucleation [1], in which cells need to be oriented spatially at a desired 3D pose with respect to a glass micropipette [1, 2]. Existing methods are capable of rotating cells in a single direction, about yaw or pitch axis, using optics [3], fluidics [4], DEP [5], and optoelectronic tweezers [6]. By constrast, our method solely uses DEP for dual axes rotation. Accordingly, our biochip differs from existing micro-devices in that it integrates six AC power excited electrodes, which are arranged on the bottom substrate and vertical walls, and controlled with voltage amplitude and phase to achieve yaw and pitch axis rotation separately. Previously, we have optimised the design of this biochip and demonstrated the dual axis rotation principle through finite element analysis methods. Thereby, yaw axis rotational electric fields are generated by solely activating vertical electrodes and pitch axis rotational electric fields by activating bottom electrodes with AC and grounding one opposite pair of vertical electrodes. Here, we report our latest progress in fabrication of a proof-of-concept biochip and the experimental data for bovine cell rotation. Figure 1 illustrates the biochip on a glass slide. Four sidewall vertical electrodes are used to induce yaw axis rotation; while the two bottom electrodes, together with the sidewall electrodes, are used to induce pitch axis rotation. Here, the novelty lies in sharing the sidewall electrodes for pitch axis rotation, leaving an open top to the biochip and providing access for the micropipette – a benefit for subsequent cell manipulation. Device fabrication involves soft lithography for transparent indium tin oxide (ITO) bottom electrodes and micro-milling for brass sidewall electrodes.

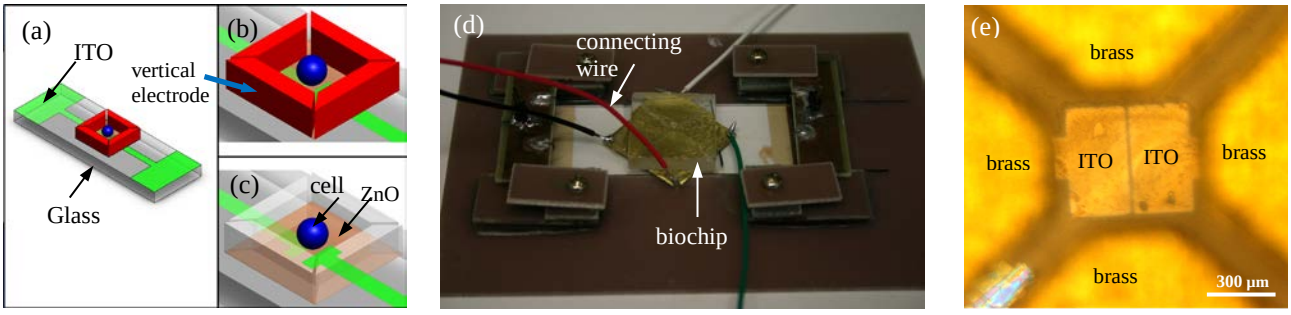

Figure 1: *DEP biochip platform design. (a) Overview of the 3D assembly of the biochip Note vertical electrodes are isolated from the bottom ones via an insulator layer (ZnO). (b) Four isolated vertical electrodes of height 500 µm forming a chamber with a cell inside. (c) Two bottom ITO electrodes underneath the chamber, separated by a 30-µm gap. (d) Fabricated biochip with electrical connections. (e) Close-up view shows aligned vertical electrodes on bottom ITO. The chamber workspace comprises a 750-µm square area. Dimensions are not to scale in a-c.*

DEP-BASED 3D ROTATION THEORY

Figure 2 shows the AC potential schemes for rotations in yaw and pitch respectively. Through numerical simulations, using the AC potential scheme in Fig. 2a, we found a rotational electric field is generated about the yaw axis. This induces the yaw axis rotation of a dielectric particle. Likewise, using the AC potential scheme in Fig. 2b, a rotational electric field is generated about the pitch axis, which induces the pitch axis rotation. According to established DEP-based rotation studies, the DEP torque that induces particle rotation can be formulated as [1]:

$$\mathbf{\Gamma}_{DEP} = -4\pi\epsilon_m R^3 \,\mathrm{Im}[f_{cm}]\nabla|\mathrm{E}|^2 \quad (1)$$

where ϵ_m is the medium permittivity (a frequency-dependent complex value), R is the radius of the particle, Im[] stands for the imaginary part of a complex variable, E is the complex electric field vector, and f_{cm} is the Clausius-Mossotti (CM) factor that characterizes the frequency-dependent dipole moment induced in a DEP electric field: $f_{cm} = \frac{\epsilon_p - \epsilon_m}{\epsilon_p + 2\epsilon_m}$ (2)

Where ϵ_p is the medium permittivity (a frequency-dependent complex value). Eq. (1) indicates that the rotation rate is determined by the electric field potential, the structure of the biochip electrodes, and the AC frequency. These factors provide us with a set of options to control speed and direction of the particle rotation, as well as particle trapping and translation by integrating DEP force effect.

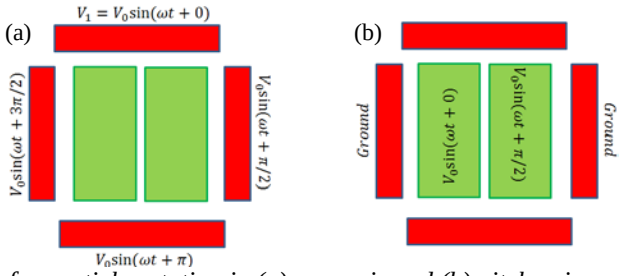

Figure 2: *AC potential schemes for particle rotation in (a) yaw axis and (b) pitch axis.*

EXPERIMENTAL METHOD

Bovine oocytes were used in rotation experiments, as our immediate goal is to enucleate them for nuclear transfer cloning applications. The biochip, as shown in Fig. 1d, has a 750 μm rotation chamber with four square tipped vertical electrodes mounted on supporting Perspex for yaw axis rotation and two bottom ITO electrodes of 250 μm width separated by 30 μm gap for pitch axis rotation. Electrodes were mounted on a glass cover slip (Fig. 1b). The entire biochip, including electrical connecting wires, was immersed in medium. For the oocyte to be suspended, the liquid solution is made up of 0.85% (w/v) dextrose and 3% (w/v) sucrose. Using a mouth pipette, a single oocyte was transferred into the device rotation chamber through a small hole drilled on Perspex. AC potentials were generated by a 40 MHz four channel arbitrary sine wave signal generator and applied with appropriate phase shifts on the vertical electrodes for yaw axis rotation and bottom ITO electrodes for pitch axis rotation, in line with the potential schemes shown in Fig. 2. Oocyte rotation was imaged in bright-field mode at 50 × objective using an inverted microscope (Nikon Eclipse 80i). Videos were obtained at 8 Hz and analyzed offline using customized image processing software (ImageJ). Angular velocity was estimated by manually picking rotating pixel point in ImageJ.

RESULTS AND DISCUSSION

Figure 3 shows yaw and pitch axis rotation image sequences. A comparison of the rotation spectrum plots between experimental and simulated results is shown in Fig. 4 and Fig. 5. For the simulation, the bovine oocyte was anatomically treated as a spherical model. Consistent with the simulation, the yaw axis rotational experimental results had only one peak in higher frequency region between 500 kHz to 10 MHz, indicating that the bovine oocytes are permanently more polarizable than the medium. The deviation in the peak value is probably attributed to errors in selection of parameters (e.g., oocyte/medium permittivity and conductivity) used in the simulation. Due to the absence of a negative rotation peak in the lower frequency region between 0-500 kHz, the Maxwell Wagner (MW) relaxation at a critical frequency of 500 kHz is eliminated. In contrast to the yaw axis spectrum, the pitch axis rotation spectrum differs from our numerical simulation result in which the rotation peak occurs at 10-100 kHz. The presented simple and affordable DEP biochip platform shows a great promise in rotating biological entities in 3D space. Besides rotation, this device can also be used to characterize cell dielectric properties, which are important to cell sorting, analysis and characterization.

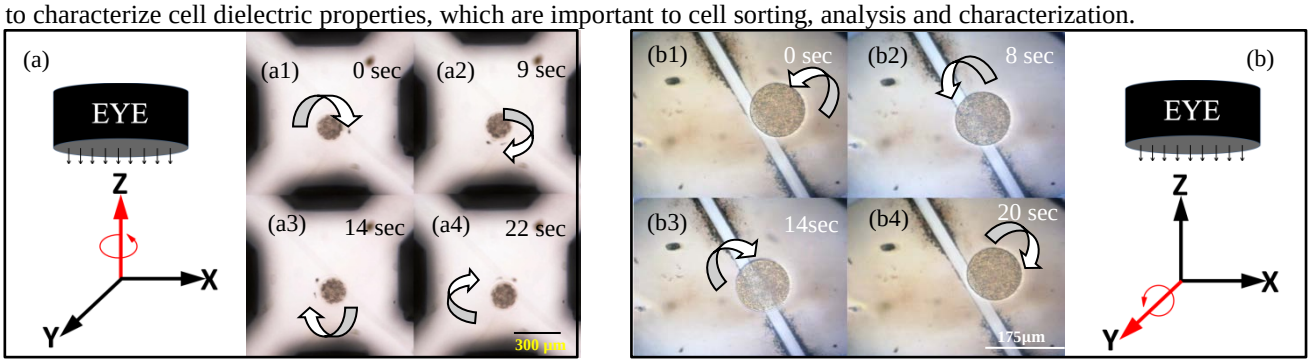

Figure 3: *Experimental results. (a). Co-ordinate system along with a single bovine oocyte undergoing yaw axis rotation at 10V, 500 kHz frequency and 90° phase shift between two pairs of orthogonally arranged vertical electrodes. Arrow shows rotation direction of oocyte. (b). Co-ordinate system along with a single oocyte undergoing pitch axis rotation at 10V, 80 kHz frequency and 90° phase shift between two bottom electrodes and grounded vertical electrodes. Arrow shows the rotation direction of oocyte rotation.*

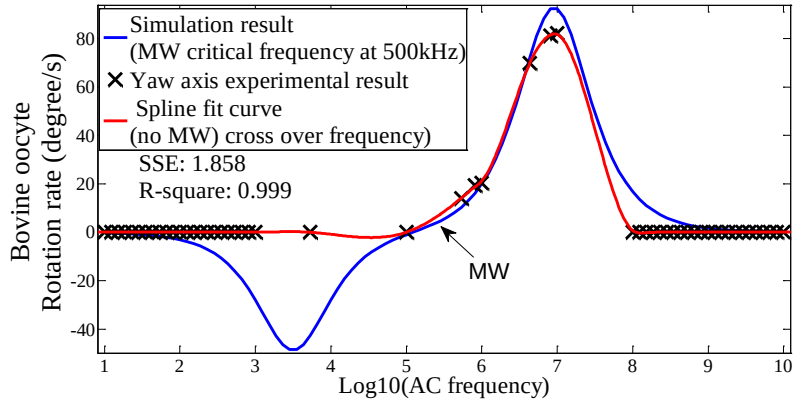

Figure 4: *Comparison of simulated and experimental rotation rates of bovine oocyte with respect to z-axis (yaw-axis rotation). Only one peak is evident from experimental results, showing bovine cells are consistently more polarizable than the surrounding medium over the frequency range. Permittivity of medium is taken as 78 along with conductivity of 3.5μS. Electric field strength is 8×10^3 V/m at 10V peak-to peak potential.*

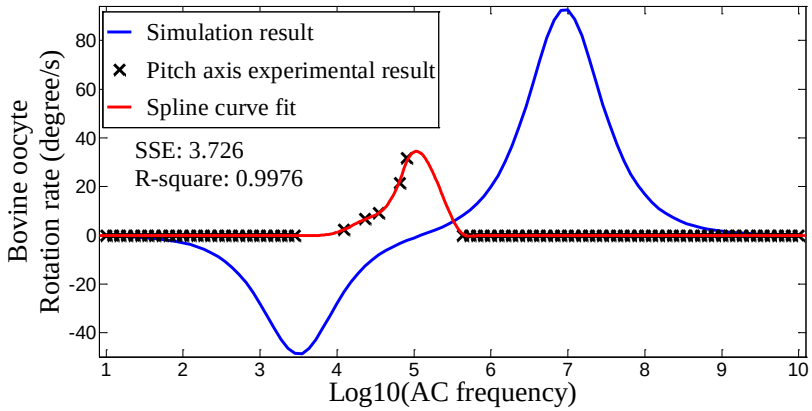

Figure 5: *Experimental rotation rate of bovine oocyte with respect to y-axis (pitch). Electric field strength is 2×10^4 V/m at 10V peak-to-peak potential.*

CONCLUSION

We have demonstrated DEP based rotation of spherically dielectric particles, such as bovine oocytes, about both yaw and pitch axes in a micro-fabricated biochip. Our findings suggest that bovine oocytes are more permanently polarizable than the surrounding medium. Pitch axis rotation is estimated to be suitable between the AC field of frequency10 kHz to 100 kHz and yaw axis rotation between 100 kHz till 10 MHz. The biochip working solely on the DEP phenomenon to rotate cells in 3D spaces can find immediate applications in automation of enucleation during nuclear transfer cloning.

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