DIELECTROPHORETIC PRESSING OF BIOLOGICAL CELLS INTO CONTACT WITH SURFACES: A MECHANISM FOR BIOPHYSICAL FLOW CYTOMETRY

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ABSTRACT
The surface morphology and mechanical properties of a biological cell provide insight into its physiological state. The interaction between a dielectrophoretically actuated cell and the wall of a microfluidic channel is monitored using an electrical approach. Pressure-driven cells generate position-dependent capacitance signatures as they flow past interdigitated coplanar electrodes. Distinct capacitance signatures are observed when cells are driven into contact with channel walls using dielectrophoretic actuation. Simulations of the equation of motion are used to estimate the experimental trajectories. Since different cell phenotypes exhibit different surface properties, their respective cell-wall interactions may form a basis for their differentiation.

KEYWORDS: Dielectrophoresis, Capacitance, Impedance, Biophysical Flow Cytometry, Microfluidics, Single-Cell Analysis

INTRODUCTION
We present an electrical approach for controllably pressing cells into contact with surfaces, and detecting the corresponding contact dynamics. Atomic force microscopy (AFM) has revealed that healthy and cancerous epithelial cells can be differentiated based on the interaction between their “brush layers” (mainly microvilli, microridges, and cilia) and an AFM probe [1]. Other extensions such as blebs appear on Chinese Hamster Ovary (CHO) cells depending on their cell cycle phase [2]. These types of cell surface extensions influence the way cells adhere to surfaces.

In MicroTAS 2009, we presented an interferometric approach for capacitively detecting the presence and dielectrophoretic (DEP) manipulation of single biological cells, that was subsequently described in detail [3]. An RF (~1.5 GHz) signal applied to a pair of interdigitated coplanar electrodes produces a non-uniform electric field in a microfluidic channel. A biological cell, which has a lower permittivity than the surrounding fluid medium, displaces the medium as it flows past the electrodes and consequently decreases the capacitance within the volume above the electrodes. In this work, we simultaneously apply a lower frequency (1 kHz – 10 MHz) potential to the interdigitated electrodes, which produces a repulsive DEP force of sufficient magnitude to press a cell into contact with the channel ceiling (Figure 1).

The cell translates along the channel ceiling under the influence of DEP, fluid flow, and the surface-to-wall interaction forces. We have observed these interactions using both optical observation and the time dependent capacitive signal.

Figure 1: Side view. A laminar fluid flow drives the cell from left to right in a microfluidic channel. When passing over the interdigitated electrodes, the cell experiences a DEP repulsion from the electrodes toward the channel ceiling. Depending on the nature of the cell surface, the cell translates slowly along the channel ceiling due to the combination of fluid flow, DEP, and contact forces between the cell surface and channel ceiling.

THEORY
The particle trajectory can be estimated by solving the equation of motion for the cell. The forces considered are DEP, drag, buoyancy, gravity and a lift force. The DEP force is calculated after first evaluating the electric field distribution in the channel by solving the Laplace equation using the finite element analysis program, COMSOL Multiphysics®. A 1 MHz, 6 Vpp voltage is applied to the electrodes, which yields a Clausius-Mossotti factor of -0.4 (repulsive force) using the sizes, permittivities and conductivities of CHO cell components noted in the literature. The medium is assumed to have a permittivity of 78 and a conductivity of 1.6 S/m. For the trajectory calculation, the cell was initially elevated 15 µm above the electrodes and given an initial velocity of 280 µm/s. In the microfluidic channel, the fluid flow is laminar with a parabolic profile. The simulated trajectory is given in Figure 2.
EXPERIMENTAL
An interferometric system described previously is used for capacitive detection and dielectrophoretic manipulation of biological cells [3,4]. The transmission line resonator is coupled to a 40 μm x 120 μm microfluidic channel. Platinum electrodes having a 25 μm width and spacing are fabricated on the channel floor and have a two-gap interdigitated configuration as shown in the insets of Figures 2-4. Cells are pressure-driven in the phosphate buffered saline (PBS) filled H-channel using an automated pump, which delivers cells across the electrodes at a velocity of 50-300 μm/s. As a cell passes over the electrodes, it generates a position-dependent output voltage that is proportional to the induced capacitance change. This “capacitance signature” is correlated with video micrographs acquired at 15 frames per second.

RESULTS AND DISCUSSION
Figure 2 shows the position versus time, measured using video micrographs, of a Chinese Hamster Ovary (CHO) cell. The initial cell velocity, region A, is estimated by fitting a line through the positions (dots) before the cell enters the electrode region. As the cell enters the electrode region (B), it rapidly accelerates toward the channel ceiling due to the repulsive DEP force. Upon contact with the ceiling, the cell slows considerably and then translates slowly until it reaches a weaker field region where the fluid flow can initiate the cell’s release. During this time, the cell velocity is controlled by the interaction between the cell and wall, the magnitude of the DEP forces, and the forces exerted by the fluid flow. When the cell exits the electrode region, it remains high in the channel in a slow laminar flow position (see Figure 1), which is confirmed by the smaller exiting slope of region C.

These interactions are also observed in the capacitive signatures. Figure 3 reveals the unperturbed and DEP-influenced capacitance signatures for a CHO cell. In the unperturbed case, the cell produces a capacitance minimum each time it flows over a gap between the electrodes (Figure 3A). With repulsive forces pressing the cell onto the channel ceiling, it slows considerably and induces much lower capacitance changes (Figure 3B). The movement of the cell results in a slow decrease in the signal as the cell moves along the surface between the electrodes and a rapid increase as it passes over the final electrode and into a region with weaker DEP fields. These phenomena have also been observed with activated human T-cells and polystyrene beads as shown in Figures 3 and 4, respectively.

Figure 2: (LEFT) Influence of DEP force on the position of a Chinese Hamster Ovary cell before (A), during (B), and after (C) it passes over the electrodes. The initial and exiting cell velocities are represented by lines, and the cell positions are noted by dots. A similar profile can be predicted by solving the equation of motion (RIGHT).

Figure 3: LEFT - Capacitance signatures due to a flowing CHO cell (A), and a DEP-influenced CHO cell (B). Inset – CHO cell is capacitively monitored while flowing left to right in a microfluidic channel over the interdigitated electrodes (dark). RIGHT - Capacitance signature of a 12 μm diameter activated T-cell experiencing an 8.5 V, 2 MHz DEP signal. T-lymphocytes were prepared in RPMI-1640 medium with 25 mM HEPES, and 1% penicillin and streptomycin - 2% BSA was added to the suspension (see [5] for details).
Figure 4: Capacitance signature of a 10 μm polystyrene bead subject to a 10 V, 100 kHz DEP signal. The output voltage is proportional to the capacitance change. Two capacitance minima occur at A and C – the electrode gaps. The capacitance maximum occurs directly over the center electrode, B.

CONCLUSION

Repulsive dielectrophoretic forces were used to press cells and polystyrene spheres into contact with a microfluidic channel wall. Cell trajectories can be estimated by solving the equation of motion. The resulting position versus time plots match experimentally observed trajectories for particles with brief channel wall contact. Capacitance signatures corresponding to the interaction between the cell surface and the wall of a microfluidic channel are clearly detected for a polystyrene sphere, a Chinese Hamster Ovary cell, and an activated T-lymphocyte. These signatures clearly differ compared with those from flowing cells not subject to dielectrophoretic forces (Figure 3A). For a given voltage and flow rate, the length of the interaction between the cell and wall (how the signal decreases) is expected to depend on the surface morphology and mechanical properties of the cell. For instance, Chinese Hamster Ovary cells contain blebs on their surfaces and are significantly softer (500 Pa) compared with polystyrene spheres (1-2 GPa). This approach can potentially be used for continuous mechanical differentiation of diseased cells and other cell phenotypes in microfluidic channels.

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REFERENCES


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