Direct Current Dielectrophoretic Characterization of Erythrocytes: Positive ABO Blood Types

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ABSTRACT

The adaptation of medical diagnostic applications into micrototal analytical systems (μTAS) has the potential to improve the ease, accessibility and rapidity of medical diagnostics. This work adapts direct current dielectrophoresis (DC-DEP) to a medical diagnostic application of sorting blood cells where an insulating obstacle is used to produce a non-uniform electric field. Initial efforts are focused on achieving separation of positive ABO red blood cells. Two dependencies will simultaneously be explored: blood type and blood cell size. Fluorescent polystyrene particles of three different sizes will be tested and compared against the separation and collection of actual blood cells into different sample bins. Further, continuous separation of red blood cells according to blood types and collection into specific bins will be explored. This developed technique is directly applicable for use in a portable device for easy and rapid blood diagnostics.

Keywords: Direct current Dielectrophoresis (DC-DEP), Red blood cells, Antigens, ABO blood type, Microdevice

1 INTRODUCTION

The use of microfluidics in channels and chambers whose cross-sectional dimensions are microns in scale [1] is directly amenable to analysis of biological samples for medical diagnosis. Such systems are commonly called μTAS or micro Total Analytical Systems with wide analytical applications such as biomedical devices, tools for chemistry and biochemistry, and systems for fundamental research [2]. Microdevices have been fabricated out of silica or glass, but thermal plastics such as polymethylmethacrylate (PMMA), polyolefins, polythylene terephthalate, carbonate or elastomers such as poly(dimethylsiloxane) (PDMS) are also widely used [2]. Such microdevices commonly utilize electrokinetics to move analytes. One specialty electrokinetic tool, dielectrophoresis (DEP), utilizes a spatially non-uniform AC electric field to exert forces on polarizable particles or cells. Dielectrophoresis has a number of advantages over linear DC electrophoresis. Electrophoresis works for surface charged particles in DC electric fields whereas DEP enables precise manipulation of polarizable particles with widely varying electrical properties. Further, dielectrophoresis is expected to play a major role in medical microdevices due to operational simplicity, low voltage electric fields, small sample volumes, and would not require skilled medical technicians to operate thus enabling device portability. The electric field strength employed for dielectrophoresis suits biological specimens as advanced by Herbert Pohl in his book "Dielectrophoresis: The behavior of neutral matter in non-uniform electric fields" published in 1978 [3]. In this book two main components were identified which contribute to the nonlinear DEP force: AC electric fields and spatial nonuniform electric field density. In AC dielectrophoresis, frequency dependencies as well as field strength dependencies are utilized to manipulate particles in precise fashions.

Direct current dielectrophoresis (DC-DEP) has been explored recently and only utilizes the spatially non-uniform electric field component, thus facilitating motion of cells. Spatial non-uniformities in the electric field are created using various insulating obstacles strategically positioned in lab-on-a-chip device channels through which a DC field is passed. The benefits associated with using an insulating obstacle were noted by Kang et.al. [4] and can be summarized as below:

1. Insulators are less prone to fouling than electrodes embedded in microdevices,
2. No metal components are involved which reduces the complexity of fabrication of devices,
3. Structure is mechanically robust and chemically inert,
4. Gas evolution due to electrolysis around the metal electrodes is avoided inside the channel.

Initial work in DC-DEP, conducted by Cummings and Singh in 2003, contained an array of insulating posts where two operating regimes were observed, namely streaming and trapping DEP [5]. Insulating posts were adopted by Lapizco-Encinas et.al. (2004) to concentrate and sort live and dead *E.coli* [6]. Efforts to explore other insulating materials for electrodeless DEP included cyclo-olefin polymers by Mela et.al. in 2005 [7], and an oil droplet obstacle [8] in 2006. Thwar et.al. (2007) demonstrated dielectrophoretic potential wells using pairs of insulating oil menisci to shape the DC electric field [9].

The extension of DC-DEP to red blood cell analysis is novel. Previous research by the authors on blood cell

behaviors in AC dielectrophoresis [10, 11] showed measurable spatial separation for A^+, B^+, AB^+, and O^+ red blood cells likely due to the blood type antigen expressed on the membrane surface.

Blood type in humans is determined based on the antigens expressed on the red blood cell membrane. Blood typing is a life-essential step prior to blood transfusions. The antigens on the surface of donor blood must match the receiver's blood type or adverse immune responses can cause death in the recipient. After accidents, natural disasters, wars and terror attacks blood transfusions comprise a time sensitive, essential, and important aspect of stabilizing victims to save lives. However, current blood typing technologies require time, a clinical environment, antibody assays for each blood type, and medical technological methodologies, which are not readily portable to emergency sites. This paper analyzes a novel technique of using DC dielectrophoresis as a tool for distinguishing the positive blood types of the ABO system (A^+, B^+, AB^+, O^+). Dielectrophoretic characterizations in lab-on-a-chip devices would be far more portable than current assay techniques.

In DC-DEP, successful separation of cells into bins is dependent on the deflection from an insulating obstacle (Figure 1).

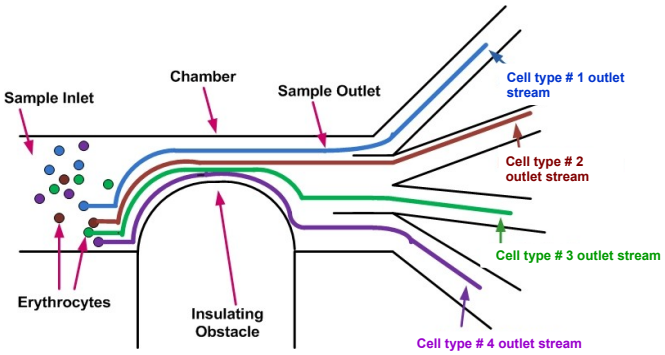

Figure 1: Insulating obstacle in a channel immediately followed by channel bifurcations for sorting.

Y. Kang etal. (2007) [12] showed that the DC-DEP force acting on a cell is proportional to cell size. Therefore, two dependencies will simultaneously be explored: blood type and blood cell size. This is possible because blood type antigens are expressed only on the red blood cells (erythrocytes) while whole blood is comprised of three different cells ranging in size from 3 μm to 12 μm: platelets, erythrocytes, and leukocytes. This technique is directly applicable for portable blood diagnostics.

2. THEORY

In DC dielectrophoresis, an insulating obstacle creates a non-uniform electric field. The resulting dielectrophoretic force acts on a polarizable particle causing it to move within the electric field gradient. This movement is governed by the particle's polarizability relative to the surrounding medium's polarizability [13]. The non-uniform field force can be derived from the net dielectric force:

$$F = (p \cdot \nabla)E \qquad (1)$$

Where p, the dipole moment vector can be broken down into particle effective polarizability, α, volume, v, of the particle and the applied electric field, E ($p = \alpha v E$). The Claussius-Mossotti factor, α estimates the effective polarizability term for spherical particles [3, 13] and is a ratio of complex permittivities $\tilde{\varepsilon}$, and is given by $\tilde{\varepsilon} = \varepsilon - i\,\sigma/\omega$, where 'ω' represents frequency, 'ε' the dielectric constant and 'σ' the electrical conductivity of the medium. The imaginary part $(i\,\sigma/\omega)$ is out of phase with the dielectrophoretic field and does not affect dielectrophoresis as it depends on the real part of the Claussius-Mossotti factor. In case of DC electric field, when there is no frequency component involved, DC-DEP can be estimated as the residual of Claussius-Mossotti factor when frequency approaches zero resulting in a dielectrophoretic force given by,

$$F_{DEP} = \frac{1}{2} v \frac{\sigma_p - \sigma_m}{\sigma_p + 2\sigma_m} \nabla E^2 \qquad (2)$$

As a result of this dielectrophoretic force phenomenon, many configurations and operating conditions are possible.

Previous work by the authors suggest that dielectrophoresis of red blood cells depends on blood type [11]. Diluted whole blood was tested at a sinusoidal AC frequency of 1 MHz with an electric field strength of 0.025 $V_{pp}/\mu m$. Dielectrophoretic movement of each blood type followed distinct trends with time as quantified with four parameters: total cell count, vertical movement, horizontal movement, and distance. All parameters revealed an attenuated O+ response that was also verified statistically at a 95% confidence interval. This might be due to the lack of functionalized antigens on type O membranes [11].

In this work, a DC field is used to generate an electric field gradient instead of the previous AC field. This dielectrophoretic field is generated inside a microchannel using a DC source and a rectangular obstacle fabricated in the channel. A spatially dense non-uniform field is created near the obstacle as the DC field lines diverge around the obstacle. Since DC-DEP forces the particle away from the high field density regions, it experiences a repulsive force when it moves around the corner of the obstacle, thus facilitating particle motion according to its polarizability.

3. MATERIAL AND METHODS

The parameters changing in this research are: voltage of the DC source, device dimensions, blood type, and time of blood storage. Blood samples will be analyzed in the

dielectrophoretic field on day 0, 1, 3 and 5 in storage at 5°C. The important steps in the process are device design, microdevice fabrication by soft lithography, experimentation (including microsample preparation), image analysis and quantification via total cells in each bin.

3.1 Red blood cells

Effective polarizability depends on the permittivity as discussed in the theory section. Charged proteins and cytosol molecules present in the cell membrane impact the ability of a cell to conduct charges and cell membranes impact the ability of charges to penetrate the cell [3, 14]. Previous researchers have shown that cell dielectric properties depend on cell shape and structure [15] thus enabling DEP forces to spatially separate cells [16]. Red blood cell membranes are nonconductive ($\sigma \leq 1$ μS/m) [17] while the interior is conductive ($\sigma = 0.53$ to 0.31 S/m) and varies due to hemoglobin and cytoplasm molecules [18]. RBCs are biconcave in shape, 6 to 8 microns in diameter, and change in response to solvent conditions, pH and temperature [19].

Human blood types are classified based on membrane surface antigens and blood plasma antibodies [20]. Type A blood expresses antigen A, Type B blood expresses antigen B, Type AB blood has both A and B antigens while Type O blood has neither antigens [20]. The A and B antigen differ only in their modifications with the side chain whereas the backbone remains the same. The A antigen terminates in an α 1,3 – linked N-acetylgalactosamine while B terminates in an α 1,3 – linked galactose. The presence or absence of the Rhesus (Rh) factor antigen determines the positive (present) and negative (absent) blood types [21]. The 8 ABO / Rh blood types are A^+, B^+, AB^+, O^+, A^-, B^-, AB^-, and O^-.

3.2 Device Design

Device designs were drawn in AutoCAD (Autodesk, Inc) and printed on transparencies with a resolution of 32,512 dpi (Fine-Line imaging, Inc.). The design consisted of one input (300 μm wide) and 4 output channels (100 μm wide). A rectangular obstacle was positioned in the input channel 250 to 1000 μm prior to the bifurcation to the four outlet channels. The rectangular obstacle was varied from 100 to 150 μm in width and 200 to 250 μm in height. The rectangular obstacle is shown in figure 2 (a) and the complete microdevice design is shown in figure 2 (b).

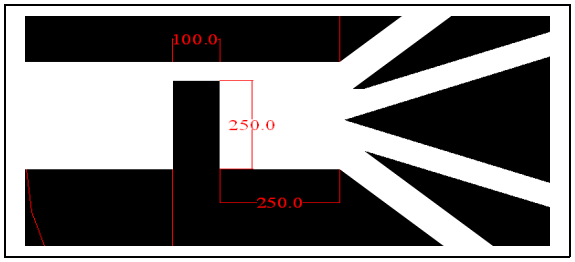

Figure 2 (a): Rectangular obstacle design

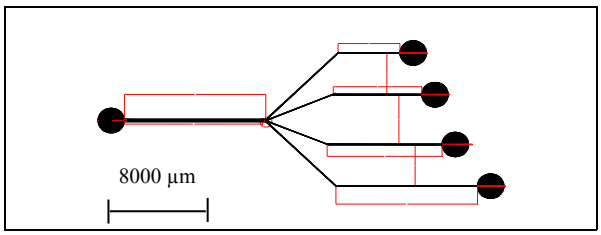

Figure 2 (b): Microdevice with input and output channels

3.3 Fabrication

The device was fabricated as a lab-on-a-chip with a fluid injection port and four outlet ports. This microdevice consisted of a glass slide and lithographically patterned microchannels of poly(dimethylsiloxane) (PDMS).

Standard techniques of soft lithography were used to pattern the microdevice [2]. Some modifications included increasing the exposure time to UV light from 2 to 5 minutes to get the smallest features like the rectangular obstacle on the SU-8 (negative photoresist) spin coated Silicon wafer of 50 μm thick. The SU-8 manufacturer, Microchem, suggests rinsing patterned silicon wafer with isopropyl alcohol (IPA) followed by air/N_2 drying to stop development, but this procedure yielded white residue. Microchem characterizes this residue as undeveloped SU-8 photoresist and recommends further immersion in the developer solution. Li suggests using Dynasolve 185 (Dynaloy LLC) [22] to eliminate residues. Figure 3 shows the patterned silicon wafer.

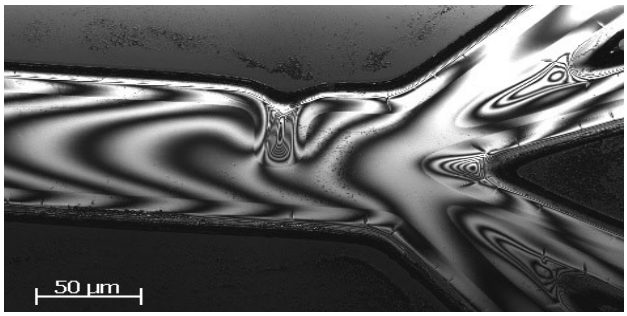

Figure 3: Patterned Silicon wafer with obstacle

PDMS was cast on the patterned silicon wafer to produce the positive relief of Figure 2 design. Sylgard 184 pre-polymer base and curing agent were mixed at a 10:1 ratio, mixed to a milky consistency, and poured onto the patterned silicon wafer and degassed for 2 hours inside a vacuum chamber to remove suspended bubbles. The degassed PDMS assembly was cured in a 65°C oven for 24 hours. The PDMS layer was peeled from the patterned silicon wafer and sealed onto a glass slide. Biopsy punches were used to create holes for inlet and outlet ports which accommodated tubing for pumping to the PDMS microdevice. Irreversible sealing can occur after UVO (UV, ozone) treatment [2]. Covalent bonds between the glass slide and PDMS can withstand pressures of 30 to 50 psi [2].

3.4. Operation

Fluorescent polystyrene particles (Bangs Laboratory, Inc) of three different sizes 2.28 μm (yellow, 540 or 600 nm), 5.49 μm (red, 660 or 690 nm) and 10.35 μm (green, 480 or 520 nm) roughly corresponding to platelets, erythrocytes and leukocytes respectively were selected for initial analysis. These beads will be tested as a size dependence reference and compared against the separation and collection of actual blood cells into different bins in the microdevice shown in figure 2.

Whole blood will be obtained via venipuncture by trained phlebotomists, drawn into vacutainers (Becton Dickinson) containing 1.8 mg K_2 EDTA per mL of blood, and stored at 4ºC in a Biosafety Level 2 refrigerator. Whole blood will be diluted 1:60 with 0.14M PBS (0.1 S/m) and introduced via ports to the microdevice mounted on a microscope. A DC power supply will deliver a voltage of 1125 V; corresponding to 0.025 V_{pp}/μm electric field strength. High-resolution video microscopy is used to record bright field video of cell motion and counts within the microdevice. Each experiment runs approximately for 5 minutes followed by image and cell density analysis.

4. RESULTS AND DISCUSSION

Cell movement is recorded between the rectangular obstacle and the bifurcation point. Cell counts are tallied in each exit port at the completion of the run for each sample. Experiments are run over day 0, 1, 3 and 5 of blood storage for each blood type [23]. Total cell counts will be tabulated after the experimental run (4 minutes) to know the percent of cells travelled to each exit port. The port having the maximum cells would likely correspond to a specific blood type. From previous AC dielectrophoresis studies by the authors, type A had the maximum deflection followed by type B, where as type O had an attenuated response in the nonuniform electric field. Type AB had deflections ranging between type O and types A and B. On this basis, authors are predicting the same pattern of deflection to be followed while the blood types separate at the bifurcation point into channels. This deflection pattern is mostly due to the existence of antigens on the membrane surface.

5. SUMMARY

The use of microdevices for blood typing can aid in increasing the speed and efficiency of emergency medical diagnosis. DC -dielectrophoresis shows potential for this purpose. This work provides evidence that dielectrophoretic responses are influenced by the expression of antigens on the surface of cells, which has prospects for future studies of the dielectrophoretic behavior of other biological cells. The current work is limited because it does not account for all the antigens on the blood cell surface, only ABO antigens are involved in this study.

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