Design and Fabrication a Microfluidic Device for Fetal Cells Dielectrophoretic Properties Characterization

Guolin Xu 1,2, M. B. Chan 2, Charles Yang 2, P. Sukumar 3
M. Choolani 3 and Jackie Y. Ying*4

1 Institute of Bioengineering and Nanotechnologies, 31 Biopolis, Way, The Nanos, #04-01, Singapore 138669
2 Nanyang Technological University, Singapore. 16 Nanyang Drive, Singapore 637722
3 National University Hospital, Singapore. 10 Medical Drive, Singapore 117597

E-mail: jyying@ibn.a-star.edu.sg

Abstract. The present work presents a microfluidic device with interdigitated microelectrode and microchannel for fetal nucleated red blood cell dielectrophoresis properties characterization using crossover frequency method. To obtain the electric field and its gradient along the microchannel, simulation study was done by using MAXWELL™ software. Results show maximum electric field and gradient are obtained near the electrode edge and they are affected by electrode width and the electrode gap. The crossover frequency should be obtained by keeping the cell moving near the electrode edge. The device has been successfully used in fetal cell characterization with better than 1KHz frequency repeatability, which is about 2% of the measured crossover frequency.

1. Introduction
Separation of rare target cells from a cell mixture is always a challenging process. For example, in prenatal diagnosis of genetic abnormalities, noninvasive diagnosis method is preferred. Fetal nucleated red blood cell (FNRBC) is the most promising candidate for the achievement of this goal. But the concentration of nucleated red blood cell in maternal blood is extremely diluted (1 per 10^6 maternal cells), any attempt to analyze these cells requires previous sorting or enrichment to avoid the contamination of maternal cells [1]. Due to non specificity of the antibody-antigen for the fetal cell, current antibody-antigen based cell isolation techniques are not suitable.

Dielectrophoresis (DEP) is an electrokinetic movement of neutral particles induced by polarization in an asymmetric electric field. Different dielectrophoretic manipulations can be applied into cells based on the differences in their dielectrophoretic properties. DEP will offer great potential for cell discrimination and isolation for the FNRBC without the use of antibody-antigen. DEP has been used in cell separation and manipulation of biological objects such as living cells and dead cells [2]; white blood cells and the subpopulations [3]; separation of cancer cells from normal cells [4] and etc. For manipulation and separation of the target cells, the dielectrophoretic properties of the cell should be obtained so the separation scheme, such as conductivity of the cell suspending buffer and the separation frequency can be determined [5].
In this paper, we present a microfluidic device for the FNRBC dielectrophoresis characterization. The device contains an interdigitated electrode system and a PDMS molded microchannels. The microelectrode is covered by the microchannel. Cell suspension is injected to the microchannel for single cell DEP characterization. For optimizing the electrode dimension, the electric field and the field gradient affected by the change of electrode width, electrode gap and electrode pitch are simulated by using MAXWELL™. The best location for cell DEP characterization is obtained. The DEP properties of FNRBC are studied in the device.

2. Device structure and theory
The structure of the DEP cell characterization device is shown in Figure 1. Microfabricated thin film microelectrode is used for generating non-uniform electric field. The microelectrode is deposited on a glass wafer. The PDMS molded cover is covered upon the electrode. The cell suspension buffer was injected to the microchannel for single cell DEP characterization.

DEP is a motion of dielectric particles caused by polarization effects in a non-uniform electric field. The time-averaged DEP force is given by the following equation [6]:

\[ F_{DEP} = 2\pi r^3 \varepsilon_m \text{Re}[f_{CM}] \cdot |\nabla E^2| \]

(1)

Where \( r \) is the radius of cell; \( E \) is the local electric field intensity (RMS). Polarity of the real part of the Clausiu-Mossoti factor \( \text{Re}[f_{CM}] \) can be calculated as:

\[ f_{CM} = \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m} \quad \text{with} \quad \varepsilon' = \varepsilon - j\sigma / \omega \]

(2)

Where \( \sigma \) represents the conductivity and \( \varepsilon \) is the absolute permittivity of particle (index p) and the suspending medium (index m).

For most of the mammalian cells, when the applied frequency \( \omega \) is high, the \( \text{Re}[f_{CM}] \) is greater than zero, and the cell will move to high electric field area, such as electrode edge in Figure 1 (b). In contrast, when the applied frequency is low, the \( \text{Re}[f_{CM}] \) is small than zero. In this case, cell will move out of the high electric field area to a weaker one. At crossover frequency, Eq. 1 should be zero since the value of \( \text{Re}[f_{CM}] \) is zero. The cell appears to be transparent to the applied field. No DEP force is induced.

Owing to the limited frequency bandwidth used in our experiment, the single shell model was implemented. This model, the cell is considered as a homogeneous, high-conductivity
sphere of radius r (the cell internal, consist of cytoplasm) surrounded by a low-conductivity plasma membrane (shell) with thickness of d as shown in Figure 2. The $\varepsilon^*_\text{int}$ and the $\varepsilon^*_\text{mem}$ are the complex permittivity of interior and plasma membrane respectively. The crossover frequency as function of specific membrane capacitance ($C_{\text{mem}} = \varepsilon_0 \varepsilon_{\text{mem}} / d$) and specific membrane conductance ($G_{\text{mem}} = \sigma_{\text{mem}} / d$) can be given as [7]:

$$f_{\text{cross}} = \frac{\sqrt{2}}{8\pi C_{\text{mem}}} \sqrt{\left(4\sigma_{\text{mem}} - rG_{\text{mem}}\right)^2 - 9r^2 G_{\text{mem}}^2}$$  \hspace{1cm} (3)

The data were analysed by minimizing the error between the measured and theoretically determined data. The following optimisation algorithm was used to determine $C_{\text{mem}}$ and $G_{\text{mem}}$:

$$\text{Min} \sum_i (f_{\text{cross,cal},i} - f_{\text{cross,exp},i})$$  \hspace{1cm} (4)

where $i$ corresponds to each experiment point and $f_{\text{cross,cal},i}$ is the calculated using Eq 4 by Mathlab™ program.

3. Experimental

3.1. Electric field Simulations

In order to obtain highly accurate crossover frequency of the cell, base on Eq. 1, the characterized electrode system must be able to provide high electric field gradient $\nabla E^2$. Figure 3 shows the partial cross sectional view of the device and its electric field distribution simulation result for a electrode with 100 µm width and 300 µm gap. When a peak-to-peak voltage of 10 V was applied, it will result in an electric field strength from $1.5 \times 10^3$ to $2.3 \times 10^5$ V/m generated along the DEP channel. Strongest electric field happens in the edge of the electrode. Figure 4 shows the electric field and the gradient above 10µm above the bottom of the measuring device, at the electrode edge where the electric field is strongest; the field gradient $\nabla E^2$ reaches its peak of $3.67 \times 10^{14}$ V$^2$/m$^3$. But the field gradient diminished rapidly. So when we do the crossover frequency measurement, the cell should move as close to the electrode edge as possible.

The DEP force and the Stroke’s viscous drag force act on a moving cell. The terminal velocity of the measured cell is reached when the DEP force acting on the cell is exactly balance in with the viscous drag force. The real component of the Clausius-Mossotti factor $\text{Re}(f_{cm})$ from Eq 2 will be zero when the applied frequency considers with the cell’s crossover frequency. Hence cell will stop moving even if it is located near the high electric field and field gradient electrode edges.
To study the effect of electrode gap on electric field strength and its gradient, a fixed electrode width of 100 µm was analyzed while varying the gap. Our simulation results show that the maximum electric field strengths and its gradient drop almost linearly with the increase of the electrode gap as shown in Figure 5. The maximum electric field strength drops from $1.4 \times 10^5$ V/m (50 µm gap) to $5.5 \times 10^4$ V/m (250 µm gap); its electric field gradients also drop from $2.15 \times 10^{15}$ V/m$^3$ to $3.47 \times 10^{14}$ V/m$^3$ in the respective gap. This suggests that the electrode gap should be small in order to obtain high electric field strength and gradient.

Electrode width effects on the electric field strength and its gradient are also studied by the simulation. Electrode gap is fixed at 100 µm in this case. As shown in Figure 6, when the electrode width increases from 50 µm to 250 µm, the maximum electric field strength drop from $1.27 \times 10^5$ V/m to $1.15 \times 10^5$ V/m; its electric field gradients drop from $1.2 \times 10^{15}$ V/m$^3$ to $8.65 \times 10^{14}$ V/m$^3$. Hence narrow electrode width is preferred.

**Figure 3.** The partial cross sectional view of the DEP device and its electric field distributions. Maximum electric field happens in the edge of the electrodes, while the minimum electric field are located above the electrodes.

**Figure 4.** The electric field and field gradient 10µm above the bottom of the channel. Maximum values appear on the edge of the electrode.

**Figure 5.** Variation of electric field strength and field gradient with electrode gap (width of electrode remains at 100 µm).

**Figure 6.** Variation of electric field strength and field gradient with electrode width (electrode gap remains at 100 µm).
3.2. Device fabrication

The electrodes were fabricated from a thin gold film deposited on glass wafer by micromachining method. In brief, a seed layer of chromium 50nm in thickness was deposited on a Pyrex glass wafer using electron beam evaporation machine (CHA) first; following 200 nm gold layer. The electrodes pattern was fabricated using standard photolithography method. In this process, the metal coated glass wafer was spin-coated with AZ7220 photoresist under 3000rpm. After soft-baking at 110 °C for 1 minute, electrode pattern on a mask was transformed to the wafer by UV light. The photoresist development was done after post-baking the wafer at 100 °C for 1 minute. The unwanted metal layers were etched away using gold and chromium enchant respectively.

The PDMS cover with a microchannel of 500µm width and 100µm depth was cased using a AZ9260 photoresist micro-mould. The micromachined mold was patterned on a 4-inch silicon wafer. In order to facilitate the separation of the cast PDMS from the mould, an anti-sticking layer (tridecafluoro-1,1,2,2-tetrahydroocty trichlorosilane, from Aldrich) was applied onto the mould by vacuum evaporation methods prior to casting. A two-part PDMS solution (Sylgard184 Silicon Elastomer, Dow Corning) was used as to cast the PDMS channels. The mixture was poured slowly into the silanized mold. The mold was then placed inside a vacuum dessicator for about one hour to release air bubbles trapped inside the uncured PDMS mixture. The whole set up was then cured inside an oven at 70°C for an hour for curing PDMS cover. The thickness of the PDMS cover was about 1.5mm. It is covered up on the electrode surface, hence a complete micro channel was formed between the electrode and the PDMS cover.

4. Results

The fabricated device is shown in Figure 7. It has an outer dimension of 22(L)×15(W)×2.5(H) mm. Figure 8 shows the experimental setup for FNRBC cell characterization.

The different structural and physico-chemical properties of a cell contribute towards its DEP response as the applied field frequency. In this work, we focused on a frequency range from 10Khz to 1MHz, where cell membrane composition, integrity, morphorphology, cell size and shape are the major controlling factors. Digital function generator (21335A from Agilent) was used to energize the device. Cell crossover frequencies under different cell suspending buffers were obtained.

The motion of the cell was observed by a CCD camera on Olimpus 370 microscope. An aliquot of the cell suspension (conductivity from 10us/m to 98us/m) was pipetted into the electrode system microchannel and the cell sample was automatically transferred to the channel by capillary force. In each test, only less than 10 cells were presented inside the device. Since the concentration of the cell is diluted, pearl chaining will not form and the crossover frequency is not influenced [7]. Cells located 100-150µm away from electrodes edge were selected for measurement. High frequency (1MHz) was applied initially to attract cell to move toward the electrode edge (Figure 9a). The frequency was reduced gradually. We kept the cell moving to avoid strong sticking and frictional force between the
cell and substrate during measuring process. With the applied frequency reducing gradually, the cell moving close to the electrode edge will stop at distance of 10-20µm from the electrode edge (Figure 9b). The frequency applied was the crossover frequency of the cell in the suspending conductivity. When the frequency farther reduced, the cell will move away the electrode edge (Figure 9c).

For fetal cell, its crossover frequency is 50 KHz when the cell suspending buffer conductivity is 15mS/m. Results show that when there is 1KHz frequency offset from the point crossover frequency, the cell located at the channel bottom with 10µm from the edge of the electrode will move at a velocity of 1-1.5 µm/s toward or out of the electrode edge. This cell’s motion can be observed effectively under a microscope.

5. Conclusion
This paper presents design, simulation and fabrication of microfluidic device with interdigitated electrode system for cell dielectrophoretic characterization. The electric field and its gradient affected by the electrode gap and electrode width were studied. The repeatability of the cell DEP crossover frequency obtained is about 1KHz. The device has been successfully used in fetal nucleated red blood cell characterization.

Acknowledgement
This work is supported by the Biomedical Research Council, Singapore, under grant 04/1/21/19/343.

References
[1] Laird Jackson, Fetal cells and DNA in maternal blood, Prenat Diagn; 23, 837-846, 2003
[2] Markx, G, M. Talary and R. Pethig. Separation of viable and non-viable yeast using dielectrophoresis. J Biotechnology. 32: 29-37, 1994.
[3] Monica Borgatti, Luigi altomare, Martina Baruffa et al. “Separation of white blood cells from erythrocytes on a dielectrophoresis based ‘Lab-on-a-chip” device, International journal of molecular medicine 15: 913-920, 2005
[4] Gascoyne P. R. C., X-B Wang, Y., Huang and F. F. Becker. Dielectrophoretic separation of cancer cells from blood. IEEE transaction on industry applications 33:3 670-678, 1997
[5] Yang, J., Y. Huang, X-B, Wang, F.F. Becker and P. R. C. Gascoyne. Dielectric properties of human leukocyte subpopulations determined by electrorotation as a cell separation criterion. Biophysical J. 76: 3307-3314. 1999.
[6] Jones T. B. Electromechanics of particles. Cambridge University Press. 1995.
[7] Ying, Huang, S. Joo, M. Duhon, M. Heller, B. Wallance and X. Xu. Dielectrophoretic cell separation and gene expression profiling on microelectronic chip array. Anal. Chem. 74: 3362-3371, 2002.