HIGH THROUGHPUT CELL SEPARATION AND FOCUS VIA DIELECTROPHORESIS BASED ON PARTICLES CHARACTERIZATION

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ABSTRACT

We present a microsystem lab chip with high throughput continuous cell separation and focus via dielectrophoresis (DEP) based on particle characterization. The experiments are demonstrated with polystyrene (PS) beads 8 µm, 25µm and HMEC-1 cells (and HepG2 cells) in suspension DEP buffer solution. Under a voltage 10 V peak- peak and frequency 100 kHz, different characteristics particles have different DEP forces. Thus, we have different deflections that mean sample is separated. Then, cells and particles are focused for collection or optical detection application.

KEYWORDS: Dielectrophoresis, Cell separation, Cell sorting, Cell focus

INTRODUCTION

Presently, dielectrophoresis cells separation tries to separating different sizes of particles (three sizes of beads) [1] or different characteristics (yeast live and dead) [2]. The focus of bioparticles in microsystem is presented by C-H Hsu [3]. We mix beads 8µm, 25µm and HMEC-1 cells (around 10 µm). Cells deflected from mixture by different characterizing their electrical properties. We can separate cells from sample. Our chip is designed as Figure 1. It has three inlets (one for sample inlet, two others for DEP buffer) and three outlets (one for waste solution, one for cells collection and one for beads). The electrode array is designed with 45^0 sloping. It includes a system for separation and a system for focus particles. The separation ability depends on hydrodynamic drag force and DEP force is shown in Figure 2.

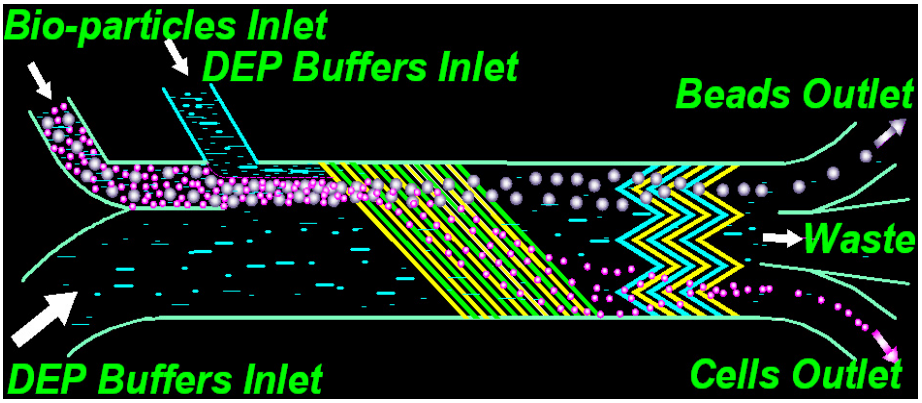

Figure 1. The principle of design

THEORY

The cells and particles in our chip is effected of DEP force and hydrodynamics force. DEP force effect on particle in microchannel can express by x direction and y direction:

$$F_{xDEP} = \pi\varepsilon_m r^3 \,\mathrm{Re}[f_{CM}]\frac{\partial}{\partial x}|E|^2 \quad (1) \qquad F_{yDEP} = \pi\varepsilon_m r^3 \,\mathrm{Re}[f_{CM}]\frac{\partial}{\partial y}|E|^2 \quad (2)$$

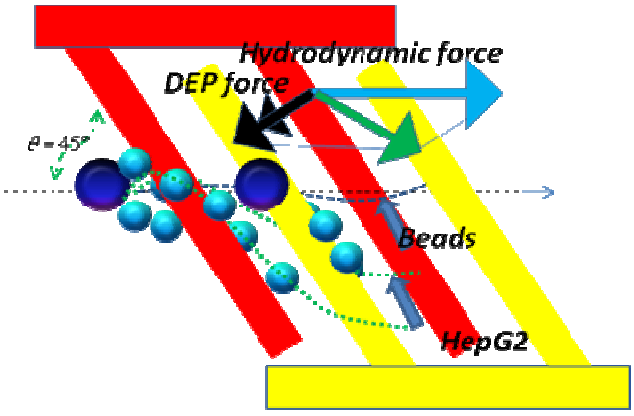

Figure 2. The principles of operation

Cell and particle motion in micro-channel effect is hydrodynamic force follow x direction and y direction :

$$F_x drag = 6\pi\eta r W_x \frac{dx}{dt} \quad (3) \qquad F_y drag = 6\pi\eta r W_y \frac{dy}{dt} \quad (4)$$

Force balance: At steady state, the DEP force is balanced by the viscous drag:

$$\vec{F}_{DEP} + \vec{F}_{drag} = \vec{0} \quad (5)$$

Thus, the velocity of cell or particle follow x direction and y direction in microchannel of our DEP device as:

$$v_x = \frac{\varepsilon_m r^2}{6\eta W_x} \mathrm{Re}[f_{CM}] \frac{\partial}{\partial x} |E|^2 \quad (6) \qquad v_y = \frac{\varepsilon_m r^2}{6\eta W_y} \mathrm{Re}[f_{CM}] \frac{\partial}{\partial y} |E|^2 \quad (7)$$

FRIBRICATION AND EXPERIMENT SETUP

The micro-fabrication process used IC fabrication to fabricate electrode: The first, Ti coating on glass wafer, lithography and wet etching, we have electrode. The second, we used soft lithography to fabricate microchannel. We coated SU8 on silicon wafer, lithography, pouring PDMS, drill hole, oxygen plasma treatment and bonding. We have the chip as is shown in Figure 3. For experiment setup, we used a function generator to create DEP force acting on the particles. We used a syringe pump to provide controlled flow through the microchannel shown as Figure 4. CCD camera from microscope to catch picture is connected with computer as is shown in Figure 4.

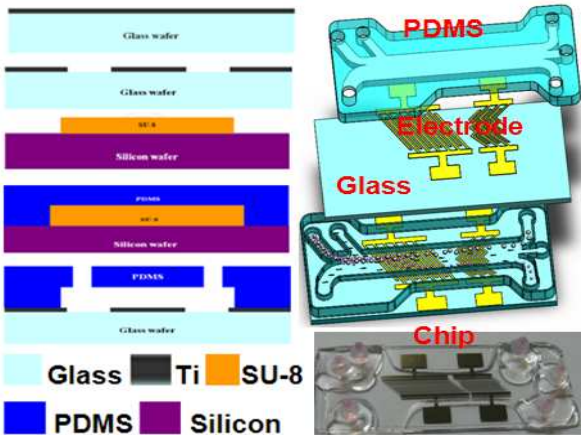

Figure 3. Schematic diagram of Chip fabrication process

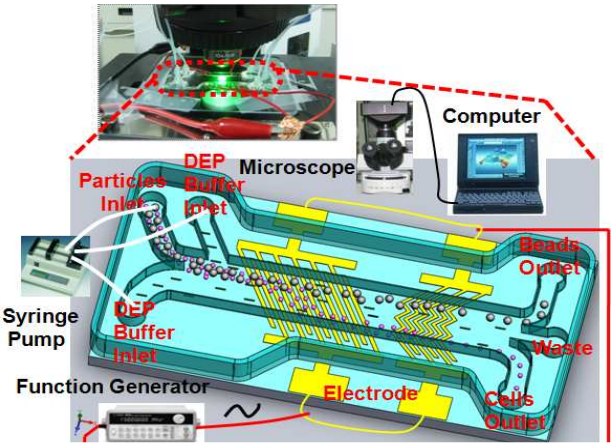

Figure 4. Schematic diagram of the experimental setup

RESULTS AND DISCUSSION

The Figure 5 illustrates cells and particles are separated and focused in our chip. To increasing high throughput, we increase flow rate on 7µL/min. The ability deflection is shown as Figure 6. The firstly, our chip works on high flow rate, and it overcome previous devices working at 4µL/min [2]. The secondly, we do experiment with cells high density in final concentration 10^8 cells/ mL compare with chips presented [4].

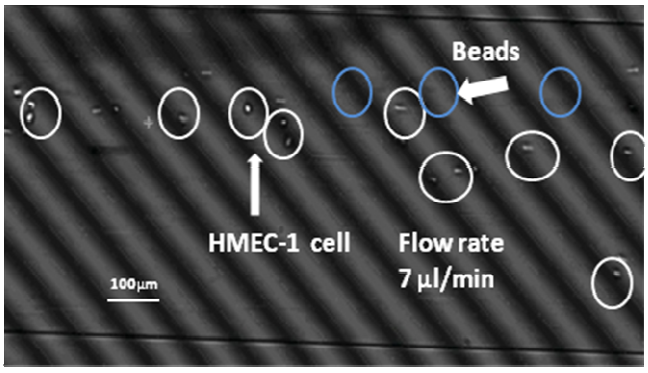

Figure 6. Illustrates cells deflected on chip with high flow rate 7 µL/min

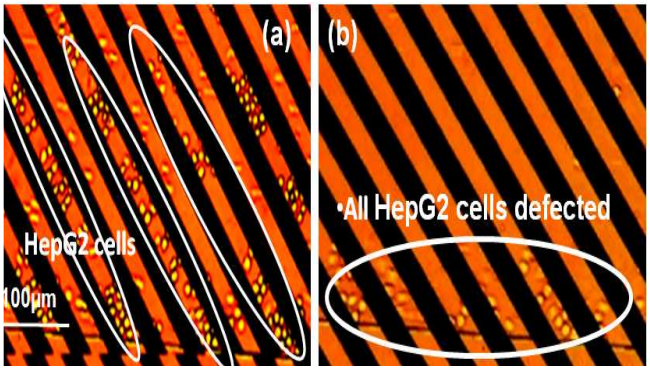

Figure 7. Illustrate deflection ability all of cells on chip with high density suspension

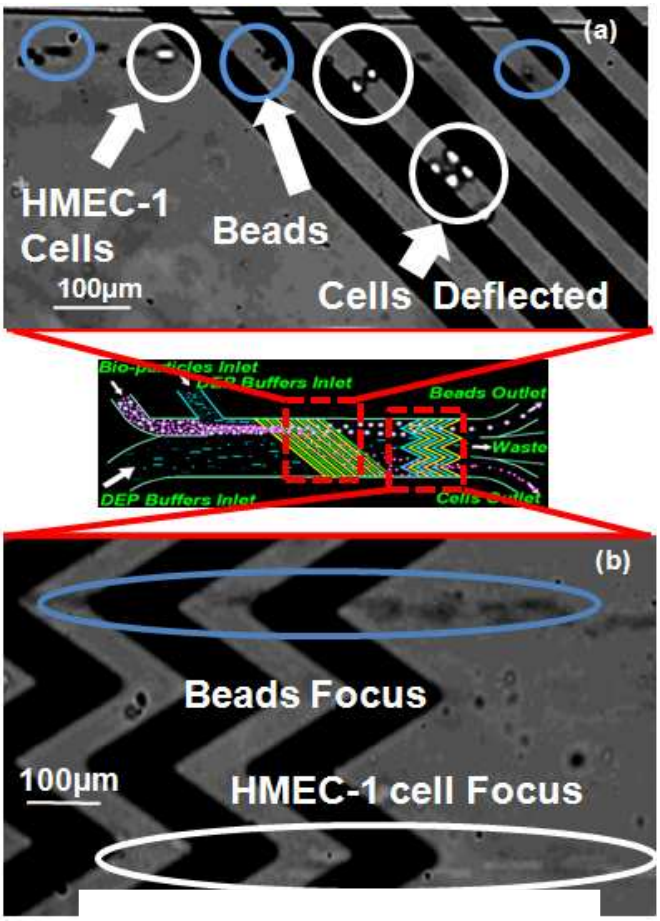

Figure 5. Illustrates cells are separated and focused on our chip.

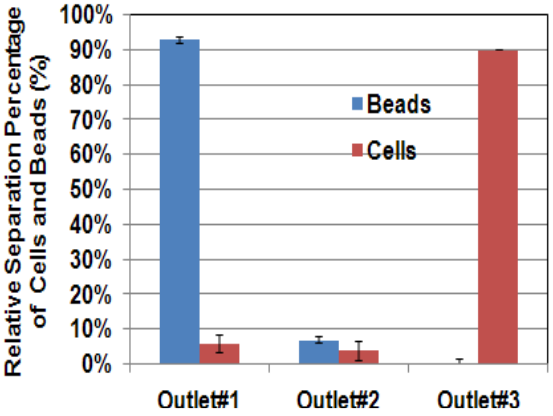

Figure 8. The separation Percentage of Cells & Beads at three outlets with flow rate 2 μl/min

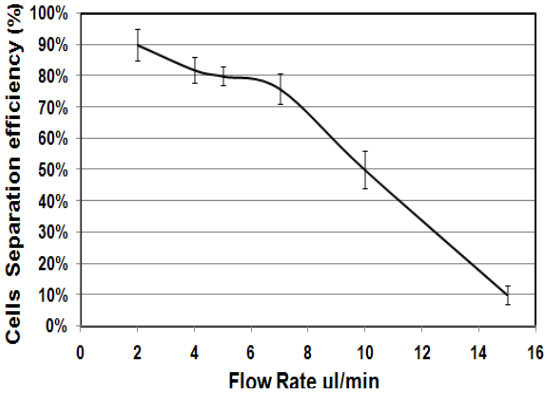

Figure 9. Cell Separation efficiency of device at 100 kHz and 10 V_{p-p} for flow rates of 2, 4, 5,7,10 and 15 mL min^{-1}.

All of cells are separated rapidly shown as Figure 7. The Figure 8 illustrates relative separation percentage of cells and beads at three outlets with 2 μL/min flow rate. The Figure 8 shows relationship cell separation efficiency with flow rate at 100 kHz frequency, 10 V_{p-p}. We want to discuss about diameter of particles. The cells have diameter around 10 μm and beads are 25 μm. Many previous publishes show that the diameter is very important. It is main factor for separating but in this chip it is dominated by one thing which makes increase ability separation. In the future, we try to get better result with better image. The test of cell viability is necessary. The factors effect to cell viability as voltage, frequency and flow rate are considered. One of good ways is exchange buffer for cell when device working.

CONCLUSION

We designed and fabricated successful a microsystem lab chip for high throughput separation and focus bioparticle via dielectrophoresis based on characterization of particles. Compare with present devices we can separate particles at high flow rate and high density of cells. The electrode array increases the ability of deflection of particles. In high velocity or hydrodynamic force larger, the particles will be deflected at further electrodes.

ACKNOWLEDGEMENTS

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