A MULTI-LAYERED MICROFLUIDIC DEVICE FOR MAGNETOPHORETIC CELL SEPARATION

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Abstract: In this paper, we present the design and experimental results of a multi-layered microfluidic electromagnetic cell separation device. Our channel consists of top and bottom layers in order to separate magnetically labeled cells in the vertical direction. Rapid separation of magnetic beads in top and bottom channel can be used in high throughput screening to monitor the efficacy and drug compounds. The experiments using the device were carried out with 4.5μm magnetic bead and magnetic labeled Jurkat cell under electromagnetic field of 1.55mT. Without the magnetic field, the magnetic labeled cells started to flow from the bottom inlet and exit out of the bottom channel outlet. In the presence of the magnetic field, the cells started in bottom channel are attracted upward by the electromagnetic field and flow through the top layered. Finally, the labeled cells flow out the top channel outlet. The separation efficiencies of the multi-layer structured microfluidic channel showed more than 95%. We found that the multi-layer structured microfluidic channel was very effective in enhancing the separation. This microfluidic channel can be potentially applied to Lab-on-a-chip system because of its attractive features such as high throughput, continuous sorting, simple and rapid fabrication.

1 INTRODUCTION

There is a growing interest of microfluidic cell separation systems as they are useful for high throughput drug screening and medical diagnosis (Inglis at al. 2004) (Pamme and Wilhelm, 2006).
The Fluorescent Activated Cell Sorter (FACS) is one of the most common methods to detect and separate cells but the bench-top volume of the device is a barrier to its miniaturization. A microfluidic device is a proven way of minimization and there have been several reports regarding the separation of specific cells in an optical microfluidic system (Wolbers et al. 2004). However, optical systems have the disadvantage of requiring external observation and are not suitable for opaque samples like blood.

A Magnetic Activated Cell Sorter (MACS) can overcome all the defects of cell sorters. It is simple to operate and is generally not affected by the electrical properties of a solution, pH, temperature or impurities (Pamme 2006). Separation of human peripheral T lymphocytes has been reported using MACS with permanent magnets and quadropole fields (Sun et al. 1998). Microfluidic MACS for HeLa cell and macrophage sorting have recently been developed (Pamme and Wilhelm 2006).

It is a generally, thought that conventional magnetophoretic separating devices produce small magnetic fields in the microfluidic channel. Also, most use mono-layered channel that separate in the horizontal direction (Kim at al. 2007).

In this research, we demonstrate a new microfluidic channel consisted of top and bottom layers in order to separate in the vertical direction. This device can get easily high degree of separation efficiency although in the small magnetic fields.

2 MATERIALS AND METHOD

2.1 Theory

There are three forces acting on a magnetically labeled cell surface: magnetic force, drag and gravity as shown in Fig.1.

Because the effect of gravity is negligible owing

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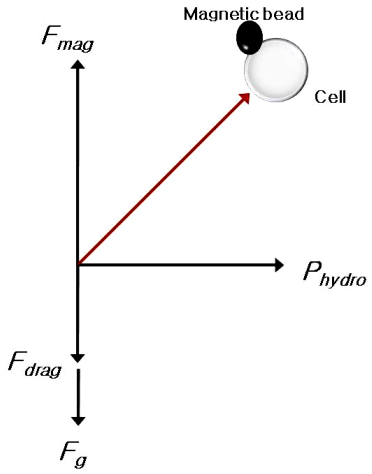

Figure 1: Three forces acting on the surface of a cell.

to the small size of the magnetic bead labeled cell (Qasem et al. 2004), the forces responsible for deflection of cells coated with magnetic beads are magnetic and drag forces. The magnetic force exerted on a magnetic bead in magnetic field can be calculated as follows (Williams et al. 1999):

$$\vec{F}_{mag} = \frac{1}{2\mu_0} V_m \Delta\chi \left|\nabla \vec{B}^2\right| \quad (1)$$

Hydrodynamic drag force is estimated according to the Stokes drag equation:

$$\vec{F}_{drag} = -6\pi\eta R \cdot \vec{v} \quad (2)$$

Finally, considering the force balance between magnetic and hydrodynamic drag (neglecting the inertial term of Newton's law), the deflection of the cell is given by (Qasem et al. 2004):

$$\Delta z = \frac{V_m \Delta\chi \left|\nabla \vec{B}^2\right|}{12\mu_0 \pi\eta R} t \quad (3)$$

In Eq. (3), V_m is a volume of magnetic beads. $\Delta\chi$ was calculated from the literature (Lagae et al. 2005). Finally, $|\nabla B^2|$ as determined by measuring magnetic fields with a gauss meter (LakeShore 475 DSP) and calculating based on the inverse square law. The deflection of the cell, Δz, generated by one magnetic bead attached on the cell surface was 4.5 μm. In most cases, the number of magnetic beads bound with cell was more than 1, so the vertical deflection should be more than 17.5 μm. We fabricated a microfluidic device to separate cells assuming that Δz is more than 17.5 μm.

2.2 Fabrication

2.2.1 Electromagnet

The microelectromagnet was fabricated using MEMS technology. The fabrication process for on-chip microelectromagnet is in Fig. 2: (a) SiO2 was deposited 1μm on a double-side-polished Si wafer by furnace and the microconductor was patterned using UV-lithography; (b) a copper microcoil, as a conductor for the microelectromagnet, was manufactured by 25μm thick electroplating with a photoresistor mold. For the electrical insulation, the dielectric layer based on polymer material (AZ 4620, Clariant, Korea) was deposited between the microcoil and the magnetic plate; (c) The polymer, as a dielectric layer, was encapsulated on the microcoil and was hard-baked; (d) The seed layer, Ti/Ni 500/3000Å, was deposited onto the dielectric layer for electroplating of the nickel plate; (e) the nickel, as a magnetic plate, was electroplated 25μm thick and (f) the PDMS microfluidic channel system was integrated.

The size of the electromagnet is 4 x 4 mm^2 and the height of the magnetic plate is 25 μm.

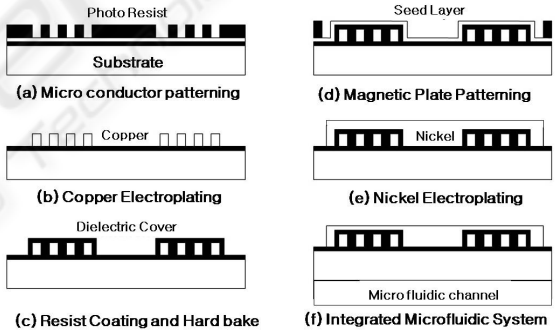

Figure 2: Fabrication processes of the microelectromagnet and microfluidic system.

2.2.2 Microfluidic Channel

A microfluidic channel was fabricated according to standard softlithography and replica molding process. The silicon wafer was washed first by methanol, followed by acetone, and de-ionized water, then a SU-8 negative epoxy-based photoresist (SU-8 2100, MicroChem Corp.) was spin-coated on the wafer. The spin-coated wafer was baked using a hot plate (95°C, 35 min) to remove unwanted area from the photoresist. The wafer was then exposed to UV light (λ = 365 nm, 60 s), baked again in two steps (65°C, 1 min and 95°C, 15 min), and developed by the SU-8 developer (Sigma Aldrich) for 15 min. The result was a 130 μm high photoresist mold. After

preparing the SU-8 mould, a PDMS gel mixture (DC 184-A:B = 9:1, Dow Corning) was poured on the wafer, the gel mixture was baked in an oven (80°C, 45 min) and detached from the mold. The PDMS microfuidic channel was finally treated with O_2 plasma and bonded with a glass substrate. The size of this device is 25mm x 14mm x 5mm and the microfluidic channel length is 17mm, the width is 150μm and the depth is 100μm.

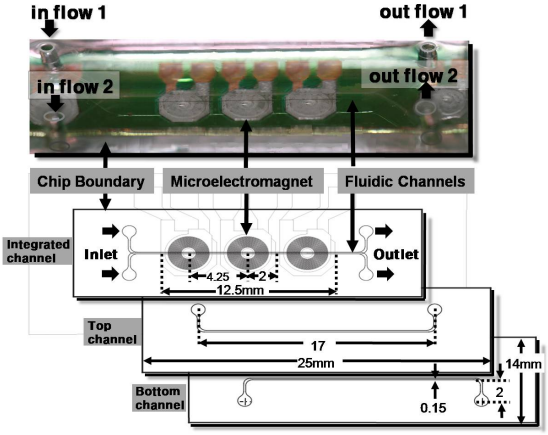

Figure 3: Illustration of the multi-layered microfluidic channel with on-chip electromagnet.

2.3 Cell Culture

Jurkat clone E6-1 cells (Organ: acute T cell leukemia/ human blood) were cultured under standard conditions (37°C, 5%CO_2) in RPMI-1640 medium comprised of 10% (v/v) fetal bovine serum (FBS) and 1% antimycotic antibiotic (all purchased from Cambrex, USA).

2.4 Sample Preparation

Dynabeads® CD3 are superparamagnetic, micro sized particles with a characteristic polymer surface for coupling with CD3 T-Cells, thus making it possible to sort out the human T cells in this experiment. Dynabeads®CD3 ($4x10^8$ bead/ml, 25 μl) and Jurkat clone E6-1 cells ($1x10^7$ cell/ml, 1ml) were incubated for 10 min at 2-8°C with gentle tilting and rotation. Finally, Jurkat cells (13 μm diameter) are bound with magnetic beads CD3 (4.5 μm diameter).

2.5 Experimental Set-up

A schematic view of the multi-layered microfluidic channel using on-chip electromagnet is illustrated in figure 4. The multi-layered microfluidic channel is a

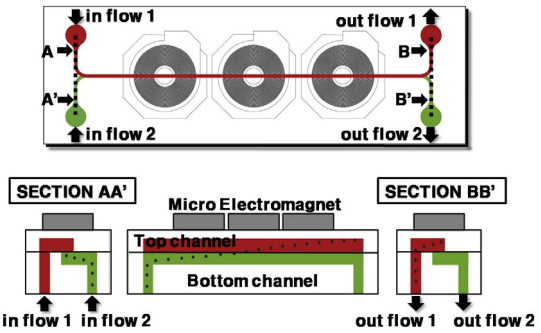

Figure 4: Schematic diagram of the multi-layered microfluidic channel.

straight type with a square cross-section. The fluid is assumed incompressible the flow will be laminar and boundary condition is the no-slip on the channel walls. The magnetic beads and cell mixture started in bottom channel at flow rate of 5μL/min are attracted upward by the electromagnetic field and flow through the top layered. Finally, magnetic beads flow out the top channel outlet.

A syringe pump (KDS scientific, CMA instruments) is connected to the microfluidic channel to supply different types of fluid through two syringes (sample solution syringe and buffer solution syringe). The syringes and channel inlets were linked by Teflon tubes (500 μm, Nano Port). The microfluidic channel was placed on an optical microscope and monitored by a CCD camera. We used a microelectromagnet of 1.55mT (Tesla) placed on the top of the main channel.

3 RESULTS AND DISCUSSION

The sample solution was flowed through a microfluidic channel and the Jurkat cells labeled magnetic beads were separated by a 1.55mT microelectromagnet at 5μL/min of flow rate. Magnetic beads are introduced to magnetic fields and then experience a magnetic field while flowing in the microfluidic channel.

Figure 6 shows comparison of the efficiencies without and with magnetic field by electromagnet. In the absence of magnetic fields, the flow of cells labeled magnetic beads is shown negligible in outflow 1 of top channel with an efficiency of 2.8%.

However, in the presence of magnetic fields, the magnetically labeled cells are dominant in outflow 1 with a high efficiency of 95.3%. It demonstrates magnetic beads were deflected and separated through outlet 1 when a magnetic field was introduced.

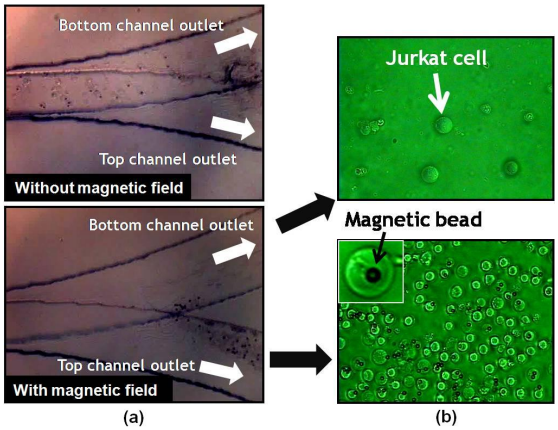

Figure 5: (a) Photographic image of microfluidic channel (b) Photographic image of Jurkat cells and magnetic beads from outlet of the channel.

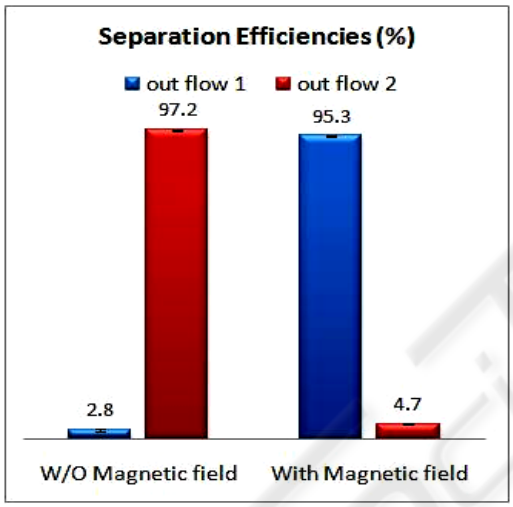

Figure 6: Experimental results of the separation efficiencies.

4 CONCLUSIONS

A new multi-layered channel for separating cells has been introduced using microelectromagnet. Our experiments demonstrate that specific cells can be separated simply using a multi-layered microfluidic channel with high efficiencies.

The efficiency of the separation by our approach was comparable with that of conventional magnetophoretic cell sorters (Bu at al. 2008) (Smistrup at al. 2005). Our results identify a new multi-layered microfluidic channel to isolate cells for drug discovery and Lab-on-a-chip system because of its attractive features such as high throughput, continuous sorting, simply and rapidly fabricated system.

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REFERENCES

Inglis, D., Riehn, R., Austin, R., & Sturm, J. 2004. *Continuous microfluidic immunomagnetic cell separation.* Applied Physics Letters, 85, 5093.

Pamme, N., & Wilhelm, C. 2006. *Continuous sorting of magnetic cells via on-chip free-flow magnetophoresis.* [Article]. Lab on a Chip, 6(8), 974-980.

Wolbers F, Andersson H, van den Berg A, Vermes I. 2004. *Apoptosis induced kinetic changes in autofluorescence of HL60 cells-possible application for single cell analysis on chip.* Apoptosis 9:749–755

Pamme N. 2006. *Magnetism and microfluidics.* Lab Chip 6:24–38

Sun L, Zborowski M, Moore LR, Chalmers JJ. 1998. *Continuous, flow-through immunomagnetic cell sorting in a quadrupole field.* Cytometry 33:469–475

Kim, H., Son, O., Kim, K., Kim, S., Maeng, S., & Jung, H. 2007. *Separation of apoptotic cells using a microfluidic device.* Biotechnology Letters, 29(11), 1659-1663.

Qasem R, Victor S, Daniel P, Chen Y. 2004. *On-chip microelectromagnets for magnetic-based bio-molecules separation.* J Magnetism and Magnetic Mat 281:150–172

Williams PS, Zborowski M, Chalmers JJ. 1999. *Flowrate optimization for the quadrupole magnetic cell sorter.* Anal Chem 71:3799–3807

Lagae L, Wirix-Speetjens R, Liu CX, Laureyn W, Borghs G, Harvey S, Galvin P, Ferreira HA, Graham DL, Freitas PP, Clarke LA, Amaral MD. 2005. *Magnetic biosensors for genetic screening of cystic fibrosis.* IEEE Proc Circuit Device Syst 152:393–400

Bu, M., Christensen, T. B., Smistrup, K., Wolff, A., & Hansen, M. F. 2008. *Characterization of a microfluidic magnetic bead separator for high-throughput applications.* Sensors and Actuators, A: Physical, 145-146(1-2), 430-436.

Smistrup, K., Hansen, O., Bruus, H., & Hansen, M. 2005. *Magnetic separation in microfluidic systems using microfabricated electromagnets-experiments and simulations.* Journal of Magnetism and Magnetic Materials, 293(1), 597-604.