STREAMLINE BASED DESIGN OF A MEMS DEVICE FOR CONTINUOUS BLOOD CELL SEPARATION

Siyang Zheng, Yu-Chong Tai

Electrical Engineering, California Institute of Technology
Pasadena, California 91125, USA

ABSTRACT

We report a new MEMS device for continuous separation of particles based on size. Unlike previous passive fluidic devices for particle separation, fluidic streamline design based on local geometry and fluidic resistance of side channels are used. The filtering effect is achieved not by physical device boundaries, but by the collection zones established by fluidic field. The separation region of the device has a small footprint of 1.5mm by 0.8mm for separation of particles in micron range. The particles are automatically collected in different exit channels after they are separated, which facilitate further sensing and processing. Like crossflow filters, particles are separated perpendicular to flow direction. The minimal feature size of the device is designed to be larger than the diameter of the largest particles, so clogging can be minimized. Solvent exchange can be accomplished for larger particles. We demonstrate separation of 5 μ m and 10 μ m polystyrene beads, as well as erythrocytes and leukocytes of human blood with better than 96% efficiency.

INTRODUCTION

Size based particle separation is a basic technique in chemical and biological analysis. Centrifugation, traditional filtration and size exclusion chromatography are incorporated into various sample preparation procedures. As devices are miniaturized to micron scale, there are efforts on separation of cell size particles, for examples, field-flow fractionation [1-3], dielectrophoretic force separation [4], magnetic separator [5], micro-fabricated filtration [6] and microstep [7]. Recently, there are a group of passive, continuous flow devices that utilizes the flow properties in micro domain, for example pinched flow fractionation [8], pillar structure based deterministic lateral displacement separation [9, 10] and channel based hydrodynamic separation [11]. Unlike active separation, these devices only require simple flow control without moving parts, signal sensing and actuation. Unlike traditional filtration plagued with membrane clogging and fouling, malfunction of devices due to clogging can be minimized.

	Erythrocyte	Leukocyte
Cell shape	Biconcave disc	Sphere
Diameter range (μ m)	5-8	7-20
Height (μ m)	1.5-3.5	7-20
Volume (μ m ³)	80 - 100	160 - 450
Count (10 ⁹ /L)	4,200 - 5,800	4.5 - 11.0

Table 1. Comparison of erythrocytes and leukocytes [12, 13].

One good application for size based particle separation is to separate erythrocytes (red blood cells, RBCs) and leukocytes (white blood cells, WBCs). As shown in table 1, erythrocytes are smaller than leukocytes with a diameter overlap. But because of their shape, erythrocytes have a significant smaller height and volume. The challenge is to design microfluidic system that

utilizes this difference and achieve separation with high efficiency. Successful separation of erythrocytes and leukocytes can facilitate leukocyte purification or downstream cell sensing and counting in a lab-on-a-chip application.

PRINCIPLE

The principle of the device under discussion is based on *separation streamline theory*. For low Reynolds number laminar flow, the centers of particles follow streamlines if there are no interactions between particles and device walls. When obstacles, such as device walls or pillar structures inside channels, are introduced into the flow, there are special streamlines that ended at the stagnation points of these obstacle structures. We call these special streamlines as *separation streamlines*, for example the labeled streamline in figure 1. The special property about the separation streamlines is that it sets up asymmetry for particle flow. Particles with centers lying on one side of the separation streamline will always stay that side if there is no particle to obstacle direct interaction. As the flow is separated by an obstacle into two flows so are the particles. The region between a separation streamline and the boundaries of the obstacles (device walls in the case of figure 1) defines a *collection zone*. The minimal width of a collection zone is the *critical separation size R*. For a particle with radius larger than R (for example the large white one in figure 1), if at upstream its center is inside one collection zone, because of particle and obstacle interaction, the center of the particle is forced to shift out of the collection zone downstream at the place where R is measured. On the other hand, a particle with radius smaller than R (for example the small grey one in figure 1), it always stay in the same collection zone. As a result, particles originally flowing in the same collection zone are physically separated based on the critical separation size R after they pass the obstacle.

DESIGN AND FABRICATION

A streamline based design is applied to the separation region. For this device, perpendicular side channels with shifted downstream edges serve as obstacles. Inside the device (figure 2), the particle flow is first pinched against main channel wall by the buffer flow so that all the particles flow in the collection zones of downstream side channels. Then they enter a separation region with an array of side channels perpendicular to main channel. Particles with radiuses smaller than the critical separation size R exit from these side channels, while larger particles are displaced by main channel wall so much that they continue to flow along it. The collection zones are defined by the channel walls and the streamlines ended at stagnation points on the downstream edges of side channels.

The critical separation size R measures the minimal distance between the separation streamline ended at a stagnation point and the upstream edge of the same side channel. R can be controlled

by local geometry of separation region and the flow resistance of the side channels. The flow pattern inside the device is simulated by solving 2D incompressible Navier-Stokes equation with FEMLAB. No-slip boundary conditions are used for the device walls. Also assumed are parallel velocity field profile at the inlet and zero pressure at the outlet. As shown in figure 3, R increases exponentially as the distance of upstream and downstream edge $\Delta\lambda$ of the same exit channel increases linearly. On the other hand, R decreases linearly as the length of side exit channels increases exponentially (figure 4).

Figure 1: Principle of device operation. Particles with diameter smaller than the critical separation size R (grey) exit from side channel. Larger particles (white) are displaced by the main channel wall so much that they can not flow into side channel. Grey scaled lines are streamlines from FEMLAB simulation. The separation streamline ended at the stagnation point is labeled.

Figure 2. Device layout: Two inlets are labeled as sample inlet and buffer inlet. Outlets are labeled as main outlet and side channel outlets from 1 to 10. Particles are pinched against main channel wall before entering the separation region. The critical separation size is controlled by side channel lengths (as shown left) and the distance between upstream and downstream edges of the same side exit channel (as shown right).

From the separation streamline theory, it's clear that the separation is binary for one geometry design, meaning there is a fixed single critical separation size and the particles are separated into two groups. To separate particles with a wide size range into more groups, geometries have to be changed. In our current design for blood cell separation, there are ten groups of side

channels and each group consists of three individual channels. For different groups, from upstream to downstream, $\Delta\lambda$ increases linearly from $0\mu\text{m}$ to $9\mu\text{m}$ and the length of the exit channels decreases linearly from $22,000\mu\text{m}$ to $2,200\mu\text{m}$. The width of the side exit channels is kept at $40\mu\text{m}$.

Figure 3. Effect of side channel edge distance $\Delta\lambda$ on minimal separation lane width R . Simulation results are based on a T shape channel structure.

Figure 4. Effect of side channel length on critical separation size R . Simulation results are based on a T shape channel structure.

The critical separation size increases from upstream to downstream as predicted by FEMLAB (table 1). Each side channel group works like a bucket to collect particles of a certain range. From side channel group number one to ten, the critical separation size R increase exponentially, which means the collection range of the bucket is fine for small particles and coarse for large particles. This is good for erythrocyte/leukocyte separation because the ratio of small erythrocytes to large leukocytes is on the order of one thousand to one.

Devices are fabricated with DRIE etched silicon molds and mounted on a glass slide by overnight baking at 80 degree Celsius. We chose PDMS (Sylgard 184, Dow Corning, MI) for the device

because of its blood compatibility and ease of use. The channel height is 20 μ m.

Side Channel Group Number	Side Channel Length L (μ m)	Edge Distance $\Delta\lambda$ (μ m)	Critical Separation Size R (μ m)
1	22000	0	2.28
2	19800	1	2.57
3	17600	2	2.88
4	15400	3	3.08
5	13200	4	3.54
6	11000	5	4.12
7	8800	6	4.79
8	6600	7	5.76
9	4400	8	7.39
10	2200	9	10.75

Table 2. Prediction of critical separation size R by simulation of 2D incompressible Navier-Stokes equation with FEMLAB.

RESULTS AND DISCUSSION

Calibration is accomplished with fluorescent polystyrene beads (Duke Scientific, CA). Flows were pumped into devices by syringe pumps (Pico Plus, Harvard Apparatus, MA) with volume flow rate of buffer flow to sample flow about ten to one.

5 μ m green beads exit mainly from side channel groups one and two and 10 μ m red ones exit mainly from side channel groups nine and ten (figure 5 and 6). Under microscope, we observed the 10 μ m beads flow in a “jumping” mode in separation region, meaning they move towards the stagnation points of side exit channels and slow down. After passing these stagnation points, they quickly flow to the next side exit channel. Assuming 5 μ m beads exit from side channel groups one to three and 10 μ m beads exit from four to ten, after normalization statistics analysis shows the separation has a high efficiency of 96% (figure 7).

Figure 5. Typical trace of 5 μ m fluorescent polystyrene beads.

Human blood cells are obtained from healthy donors and used within 48 hours. Human blood cells are prepared by sedimentation based Wintrobe method. The erythrocyte portion is diluted 1500 times with Ficoll-Paque Plus (Amersham Biosciences, Sweden). Ficoll-Paque Plus is a mixture of diatrizoate sodium and Ficoll 400. Ficoll 400 is a neutral, highly branched, hydrophilic polymer of sucrose. Traditionally it has been used as a reagent to purify lymphocytes from human peripheral blood based on density

gradient centrifugation. Its density is 1.077 g/ml, which is more close to blood cell density than aqueous saline. Diluting blood with Ficoll-Paque Plus enables us to run experiment over one hour without severe sedimentation.

Erythrocytes exit mainly from exit channel groups two and three (figure 8). To study leukocytes flow inside the device, leukocyte rich plasma is stained with fluorescent cell nucleus dye Aridine orange. Erythrocytes, which have no nucleus, do not fluoresce. Only leukocytes are stained and fluoresce. As shown in figure 9, leukocytes demonstrate a different separation profile from erythrocytes. Assuming erythrocytes exit from side channel group one to three and leukocytes exit from side channel group four to ten, a separation efficiency of 97% can be achieved after data normalization (figure 10).

Figure 6. Typical trace of 10 μ m fluorescent polystyrene beads.

Figure 7. Statistics of 5 μ m and 10 μ m polystyrene beads separation.

For the flow rate ratio used during testing, the solvent in the sample flow is observed to exit from the side channel groups one and two. So all the larger particles exit from side channel groups three and above are flow in the solvent of the buffer flow. Although in our testing, there is no difference between the solvent in the sample flow and that in the buffer flow, this can be a unique and useful property when solvent exchange for larger particles is desirable.

Figure 8. Typical trace of erythrocytes as pointed by arrow under bright field of microscope.

Figure 9. Typical trace of Acridine Orange labeled leukocytes.

Figure 10. Statistics of erythrocytes (RBCs) and leukocytes (WBCs) separation.

CONCLUSION

We have produced a MEMS device for erythrocytes and leukocytes separation. Based on the separation streamline theory, streamline based design is applied. Local geometry and fluidic

channel resistance are shown to change the critical separation size by simulation. The fabricated MEMS device demonstrates separation of $5\mu\text{m}/10\mu\text{m}$ beads and erythrocytes/leukocytes with high efficiency. The advantages of the device include high efficiency, small footprint, continuous flow, particle collection in channels after separation, minimized clogging and solvent exchange for larger particles. Further work should integrate the device into lab-on-a-chip systems.

ACKNOWLEDEG

This work is supported by NASA through National Space Biomedical Research Institute (NSBRI). The co-operative agreement number is NCC 9-58-317.

REFERENCES

- [1] J. C. Giddings, "Field-Flow Fractionation - Analysis Of Macromolecular, Colloidal, And Particulate Materials," *Science*, vol. 260, pp. 1456-1465, 1993.
- [2] Y. Jiang, M. N. Myers, and J. C. Giddings, "Separation behavior of blood cells in sedimentation field-flow fractionation," *Journal Of Liquid Chromatography & Related Technologies*, vol. 22, pp. 1213-1234, 1999.
- [3] J. Yang, Y. Huang, X. B. Wang, F. F. Becker, and P. R. C. Gascoyne, "Cell separation on microfabricated electrodes using dielectrophoretic/gravitational field flow fractionation," *Analytical Chemistry*, vol. 71, pp. 911-918, 1999.
- [4] J. Cheng, E. L. Sheldon, L. Wu, A. Uribe, L. O. Gerrue, J. Carrino, M. J. Heller, and J. P. O'Connell, "Preparation and hybridization analysis of DNA/RNA from E-coli on microfabricated bioelectronic chips," *Nature Biotechnology*, vol. 16, pp. 541-546, 1998.
- [5] M. Berger, J. Castelino, R. Huang, M. Shah, and R. H. Austin, "Design of a microfabricated magnetic cell separator," *Electrophoresis*, vol. 22, pp. 3883-3892, 2001.
- [6] S. Zheng, Y. C. Tai, H. Lin, M. Balic, R. Datar, and R. J. Cote, "Circulating Tumor Cell Capture from Whole Blood by Parylene Filter Devices," presented at microTAS2005, Boston, Massachusetts, USA, 2005.
- [7] S. Vankrunkelsven, D. Clicq, K. Pappaert, G. V. Baron, and G. Desmet, "A novel microstep device for the size separation of cells," presented at microTAS 2004, Malmö, Sweden, 2004.
- [8] M. Yamada, M. Nakashima, and M. Seki, "Pinched flow fractionation: Continuous size separation of particles utilizing a laminar flow profile in a pinched microchannel," *Analytical chemistry*, vol. 76, pp. 5465, 2004.
- [9] L. R. Huang, E. C. Cox, R. H. Austin, and J. C. Sturm, "Continuous particle separation through deterministic lateral displacement," *Science*, vol. 304, pp. 987, 2004.
- [10] S. Zheng, R. Yung, Y. C. Tai, and H. Kasdan, "Deterministic lateral displacement MEMS device for continuous blood cell separation," presented at 18th IEEE International Conference on Micro Electro Mechanical Systems (MEMS '05), Miami Beach, Florida, USA, 2005.
- [11] M. T. Yamada and M. Seki, "Hydrodynamic filtration for on-chip particle concentration and classification utilizing microfluidics," *Lab on a chip*, vol. 5, pp. 1233, 2005.
- [12] H. L. Kasdan, personal communication, 2004.
- [13] S. B. McKenzie, *Clinical Laboratory Hematology*: Prentice Hall, 2004.