SINGLE CELL MIGRATION CHIP USING HYDRODYNAMIC CELL POSITIONING

Yu-Chih Chen[1], Xia Lou[1], Patrick Ingram[2] and Euisik Yoon[1,2]

[1]*Center for Wireless Integrated Microsystems,*
Dept. of Electrical Engineering and Computer Science, University of Michigan, Ann Arbor, MI, USA
[2]*Dept. of Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA*

ABSTRACT

We report a cell migration chip which can monitor chemotaxis at single cell resolution. The chip uses a cell capturing scheme based on the difference in hydrodynamic resistance between flow paths and cellular valving. A high capture rate over 94% is achieved by optimizing the geometry of capture sites and the length of serpentine structures. After capturing, cell migration experiments induced by chemotaxis were carried out using the fabricated platform, and the behavior of each single cell was successfully traced.

KEYWORDS: Cell Migration, Single Cell, Cellular Valving, Cell Capture

INTRODUCTION

Cell migration is an essential process in angiogenesis, cancer metastasis, wound healing, inflammation and even embryogenesis. Migration occurs in response to specific external signals, such as chemicals gradients, electric fields and mechanical forces [1-2]. With this mechanism, cells can move into proper positions and enact appropriate functions in the body. There are a number of previous works reported to study cell migration in a microfluidic chip by accurately controlling microenvironments and cell conditions [3-5]. Most works, however, have loaded and cultured thousands of cells in the same channel; thus, the interaction between cells can be interfered with controlling signals. In this work, we report a cell migration chip capable of tracing cell migration at a single-cell resolution readily after cell loading. To the best of our knowledge, this is the first attempt to trace single-cell migration after positioning.

THEORY

Figure 1 shows the schematic diagram of the proposed chip and fabrication processes. Cells are loaded from a gravity flow and hydrodynamically captured at each capture site (Figure 1(C)) by cellular valving [6]. As shown in Figure 2(A), a long serpentine structure can increase the hydrodynamic resistance (R_2), which is inversely proportional to the flow rate (Q_2). With a proper serpentine length, the flow Q_2 can be smaller than the flow Q_1. Cells tend to take a low hydrodynamic resistance path (R_1), and will be captured at the capture site. Figure 2(B) shows a Skov3 cell captured at a capture site. Since the gap size (Height: 20µm, Width: 10µm) is slightly smaller than the size of Skov3 cells (average diameter of 14.1µm, SD ±3.2µm), the cells can be easily plugged into the gap to block the flow Q_1. Thus, the rest of the cells will pass through the flow Q_2, and will be captured in the next capture sites.

The migration channels (Width: 10µm, Length: 1mm) in Figure 1(A) are used to monitor the migration of cells. The generation of a diffusion profile along the migration channel was performed using food dyes (Figure 3(A)). Figure 3(B) shows the linear gradient of the diffused dye concentration observed in the migration channel, which will be adequate to induce cell migration.

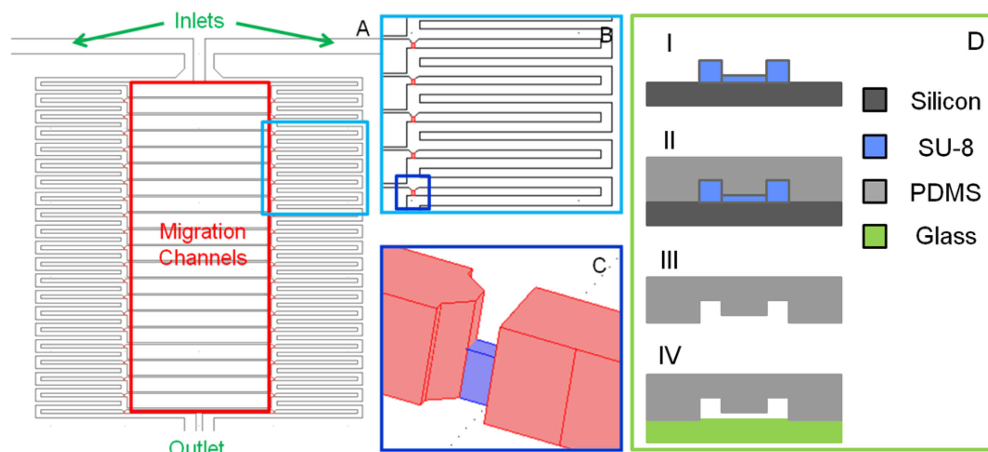

Figure 1: (A) Single cell migration chip, (B) Enlarged view of serpentine structures, (C) Single cell capture site, and (D) Fabrication process steps.

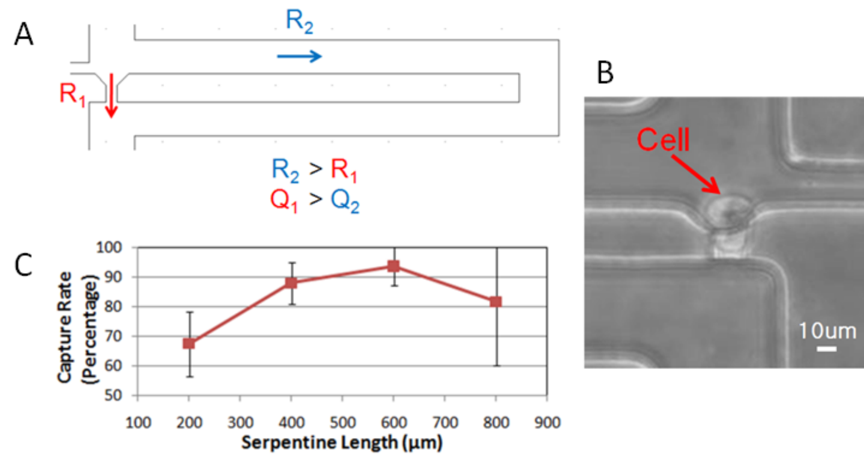

Figure 2: (A) Cellular valving principle, (B) Captured Skov3 cell at capture site, and (C) Capture rate vs. serpentine length.

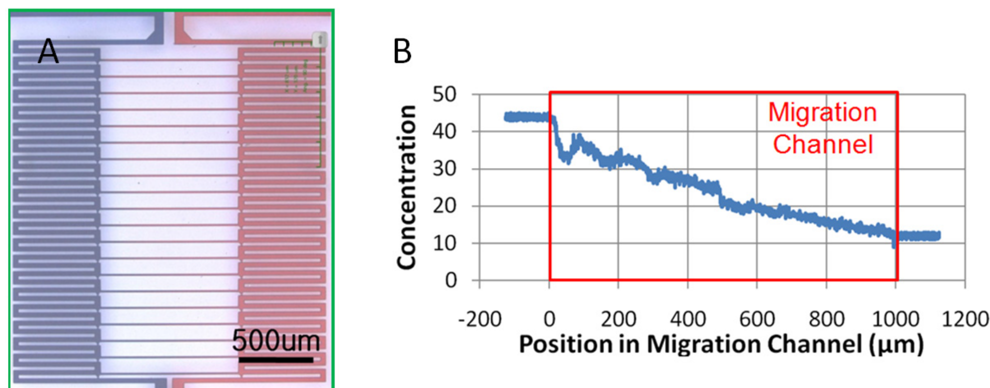

Figure 3: (A) Diffusion test using two food dyes through the migration channel, and (B) Diffused dye concentration along the migration channel.

EXPERIMENTAL

A PDMS (polydimethlysiloxane)-glass chip was fabricated by the standard fabrication process (Figure 1(D)). Two masks are used to fabricate the channel region and the gap area separately. The fabricated chip is coated with collagen to enhance cell attachment and viability.

Skov3 cancer cells are cultured with RPMI, 10% FBS and 1% penicillin/streptomycin. Before cell loading, trypsin/EDTA is used to re-suspend the cells in solution, which is diluted to 10^6 cells/mL and injected to one inlet. Meanwhile, the culture media of the same amount is added to the other inlet to balance the flow in both sides. Liquid height difference between the inlets and outlets can generate a gravity flow, and thus cells can be captured hydrodynamically. After 10 minutes, the cell solution in the inlet is replaced by culture media, and the chemoattractant is applied to the other inlet to induce migration. Then, the chip is put in the incubator, and migration behavior is observed after 24 hours.

RESULTS AND DISCUSSION

Figure 2(C) shows the relationship between the serpentine length and capture rate. Ideally, the higher the hydrodynamic resistance of serpentine path (R_2), which is proportional to the serpentine length, the higher the capture rate. However, when the serpentine length is 800um, hydrodynamic resistance of serpentine path (R_2) is large, and the flow becomes slow. In this case, cells may be stuck anywhere and may result in clogging, which can significantly degrade the capture rate. As a result, huge standard deviation of capture rates is observed in the chip with long serpentine lengths, and the optimal length can be determined. We have achieved a high capture rate over 94% (capture exact one cell) with a serpentine length of 600µm (Figure 2(C)), which is the highest among the related previous works [6-8].

In order to validate the feasibility of the fabricated migration chip, we demonstrate that chemotaxis of Skov3 cancer cells can be traced. Figure 4 illustrates the cell migration tests using HGF (Hepatocyte Growth Factor) compared with the control.

Immediately after cell loading, all the cells are positioned at the capture sites in the left side (Figure 4(A,B)). After cell loading, the media in the right side is replaced by the media with HGF of 50ng/mL, which can induce the Skov3 cell invasion [9]. After 24 hours, we observed that more cells migrated to the right side (applying media with HGF), while the control (applying the same media) did not show any distinctive migration (Figure 4(C,D)). The statistical analysis is plotted in Figure 5. The implemented chip can provide an ideal platform for cell migration study that allows accurate trace of single-cell movements without the interference of cell interactions.

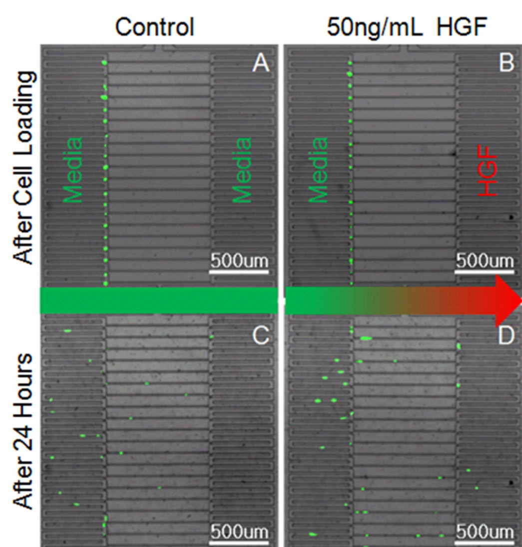

Figure 4: Cell migration test: (A,B) Cell distribution after cell loading (Cells were loaded from the left channel.), (C) Cell distribution after 24 hours with the same media in both sides (control), and (D) with HGF of 50ng/mL in the right channel.

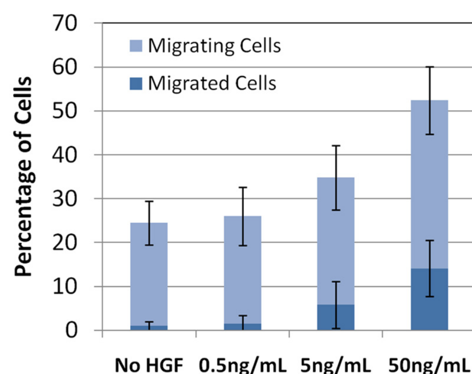

Figure 5: Relative ratio of migrated cells (to the opposite side) and migrating cells (inside the channel) vs. HGF concentration

CONCLUSION

Single cell migration chip using a hydrodynamic capture scheme is demonstrated. High capture rate is achieved by optimizing the capture site geometry and tuning the serpentine length. The feasibility of studying cell migration is proved by chemotaxis of Skov3 cells induced by HGF, and each single cell has been be successfully traced in the fabricated platform.

ACKNOWLEDGEMENTS

This work was supported in part by the Thermo Fisher Scientific Screening Technology Grant under the Center for Chemical Genomics at the Life Sciences Institute at the University of Michigan, and in part by Academic Excellence Alliance Award from KAUST. The cells are provided by Prof. Buckanovich at the Comprehensive Cancer Center at the University of Michigan.

REFERENCES

[1] F. Binamé *et al., Mol Biosyst.*, 2010, **6**, 648-61
[2] S.H. Ngalim *et al., J Oncol.,* 2010, **2010**, 1-8
[3] D. Irimia *et al., Lab Chip*, 2007, **7**, 1783-1790
[4] S. Chung *et al., Lab Chip,* 2009, **9**, 269-275
[5] D. Irimia *et al., Integr. Biol*., 2009, **1**, 506-512
[6] J.-P. Frimat *et al., Lab Chip,* 2011, **11**, 231-237
[7] A.M. Skelley. *et al., Nature Methods*, 2009, **6**, 147 - 152
[8] J. Chung *et al., Appl. Phys. Lett,* 2011, **98(12)**, 3701
[9] W. Wei *et al., Cancer Biol Ther*., 2010, **9(2)**, 79-87

CONTACT

*Y.C. Chen, tel: +1-734-5659976; yuchchen@umich.edu