MICROCHIP FILTER USING 3-DIMENSIONAL FLOW FOR RARE CELLS SEPARATION

June-Young Lee, Hui-Sung Moon, Tae Seok Sim, Minseok S. Kim, Hyoyoung Jeong, Yeon Jeong Kim, Jeong-Gun Lee, Sanghyun Baek, Jin-Mi Oh, Hun Joo Lee, Jea Chan Park, Nam Huh and Soo Suk Lee

Samsung Advanced Institute of Technology (SAIT), Republic of Korea

ABSTRACT

This paper describes a novel microchip filter device incorporating slit arrays and 3-dimensional flow that can separate rare cells with high efficiency and throughput. The proposed device has several tens of times increased throughput, and has a unique pressure distribution along the filter pore, inducing target cells to be captured and gently lined up at the end of the slit in relatively low shear stress condition. With the enhanced capture yield and throughput, the proposed device can be used as an efficient rare-cell-analyzing tool for blood-based diagnostics.

KEYWORDS

Rare cells, Filter, 3-D flow

INTRODUCTION

The separation of the rare cells from a larger population is widely used for blood-based diagnostics [1, 2]. For instance, the presence of circulating tumor cells in peripheral blood is expected to be an important indicator of the potential metastatic disease which are responsible for over 90% of cancer related deaths [3]. Among various approaches for enriching and sorting rare cells, microfluidic chip-based filtration devices are advantageous both for the versatility in the integration with other microfluidic components and for the precise, reproducible sample handling [4, 5]. However, current microfabrication capability limits the size of conventional microchip filter pore up to several hundreds of micrometers. The small length scale hinders throughput for large volume handling. Besides, large volume sample input can result in filter clogging or abrupt stress increase of the captured rare cells. Here, we report a novel microchip filter device incorporating slit arrays and 3-dimensional flow (the '3D-flow filter') that can separate rare cells with high efficiency and throughput.

DESIGN & FABRICATION

The upper inlet flow channel and the lower outlet flow channel are interconnected through the slit-shaped filter pore (Fig. 1a). The effectiveness of this device arises both from the increased filter height (or slit length) and from the unique pressure distribution along the filter pore. First, the slit length can be easily extended up to several millimeters, which increases filter throughput to several tens of times. Second, the device geometry allows the size-differentiated rare cells to be captured and gently lined up at the end side of the slit while the other relatively small blood cells (erythrocytes, leukocytes) are passing through the filter (Fig. 1b). The fluidic pressure distribution characteristics of the 3-dimensiontal flow were investigated using FEM analysis (Fig. 2). The horizontal component of the pressure along 1 mm-long slit shows maximum at the starting point of the slit (P), decreases gradually with increasing distance from (P) and shows zero at the end point of the slit (P') (Fig. 2a). Meanwhile, the vertical component of pressure along slit shows zero at the end point of the slit (P') and shows two peaks: maximum at the starting point of the slit (P) and second peak near the end point of the slit (P') (Fig. 2b). Thus, blood cells would escape from the slit during they pass through the two peak points, while the size-amplified rare cells would gently captured and lined up at the end point of the slit (P') in relatively low shear stress condition.

As shown in figure 3, the 3D-flow filter was fabricated by Glass-Silicon-Glass process to realize precise filter dimension and optical observation. Briefly, silicon and glass wafers were bonded using anodic bonding. Lapping and chemical mechanical polishing (CMP) were performed on the silicon layer. This process determined the height of the filter and 50 μm thickness of the silicon layer was remained in this study. Using photoresist AZ 4330 (Clariant Corp., NJ) as a etch mask, slit arrays were patterned on the silicon layer by deep reactive-ion etching (DRIE) process. Using the patterned silicon layer as a mask, the outer flow channel is patterned by isotropic wet etching of the glass layer in a hydrofluoric acid (HF) solution. The inner flow channel is patterned on the capping glass wafer using the same HF solution then a dry film photoresist, Ordyl BF 410 was laminated and patterned. When the sandblasting process was finished for an inlet and an outlet port, the capping glass wafer was aligned and bonded with the SOG wafer by the 2nd anodic bonding process. The fabricated 3D-flow filter microchip has an array of 700 slits (Fig. 4a), and is loaded to an automated workstation for the fluid control (Fig. 4b). The work station consists of liquid handler module, syringe pump, pipette tip cartridge, reagents holder and chip cartridge.

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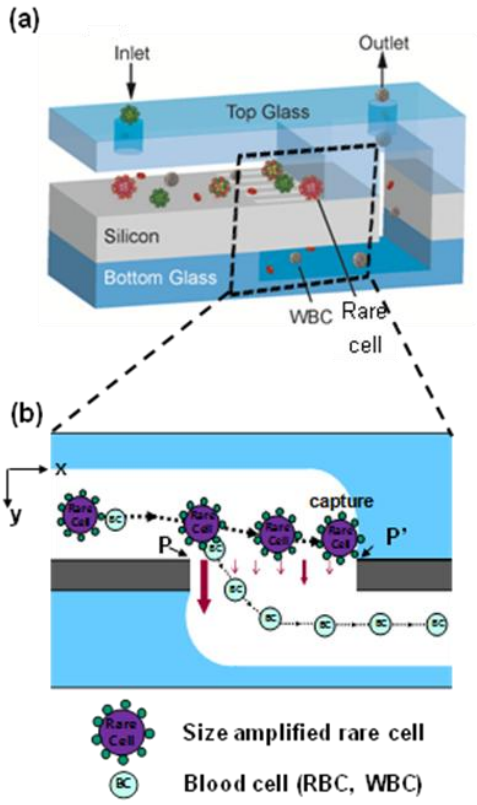

Figure 1. (a) Schematics of the proposed 3D-flow filter (b) Enlarged cross-section view around a slit

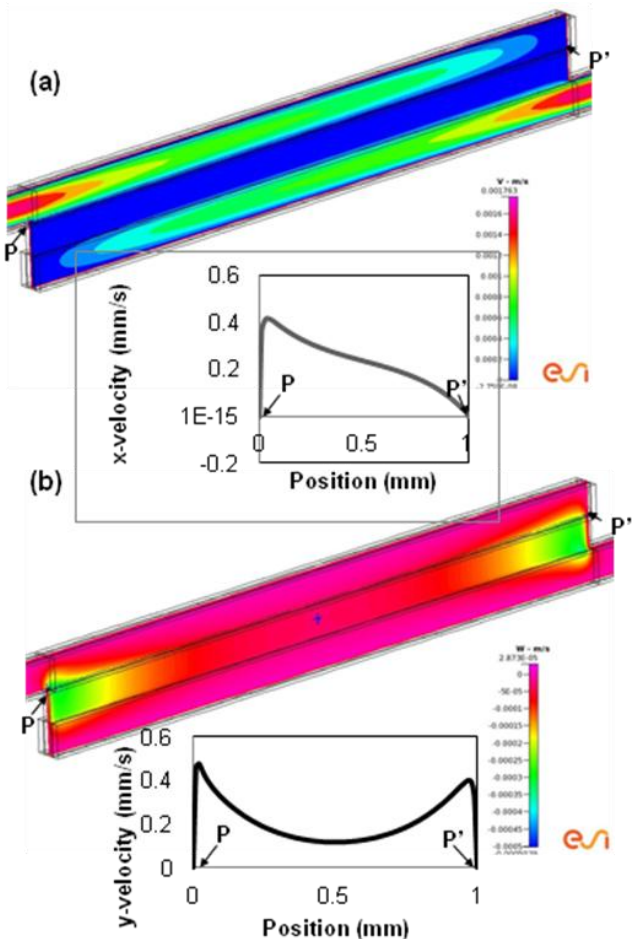

Figure 2. Pressure distribution characteristics (a) Horizontal term of the flow velocity (b) Vertical term

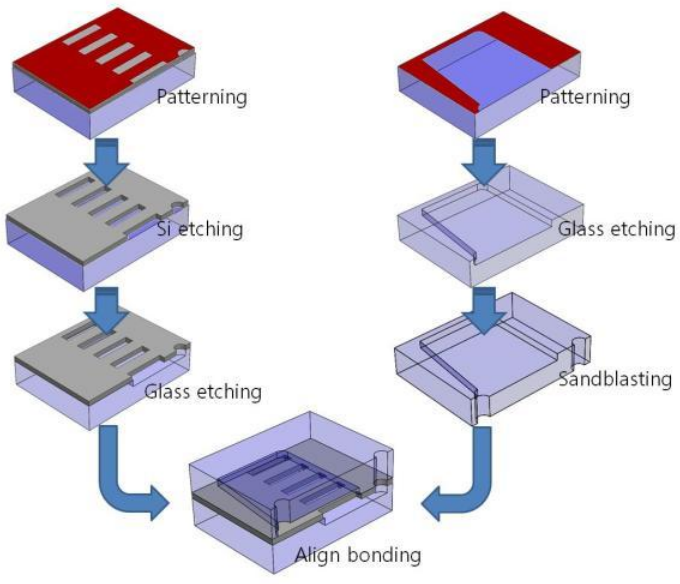

Figure 3. Fabrication process

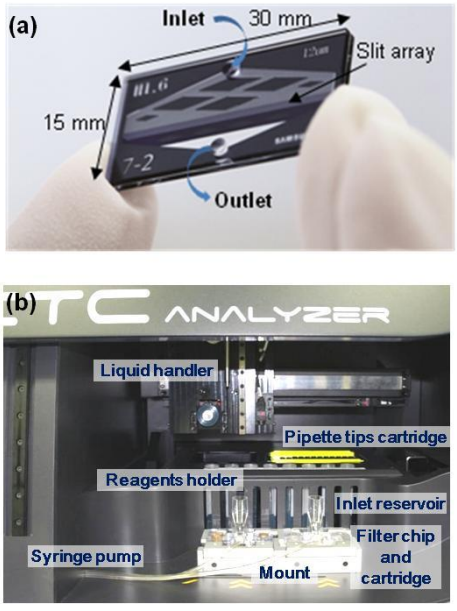

Figure 4. (a) Fabricated 3D-flow filter chip (b) Automated workstation for fluid control

EXPERIMENTAL RESULTS

To determine the efficiency of the proposed device, we spiked breast cancer cells (MCF-7) into phosphate buffered saline (PBS) at 100 cells ml-1 and captured the spiked cancer cells using the 3D-flow filter. The capture

efficiency was maintained above 90% up to the flow rate of 1 ml min^{-1}, leading us to select a flow rate of 0.1–1 ml min^{-1} for subsequent studies (Fig. 5a). To evaluate the cell capture efficiency under physiological conditions, recovery rate was investigated at various input cell numbers (Fig. 5b). MCF-7 ranging from 6, 13, 66, 133 and 664 in number were spiked into 5ml whole blood. The recovery rate achieves a minimum number of 90% with 99.9% linearity. Captured cells were identified using immunofluorescence staining, and the image showed clear discrimination of MCF-7 from WBCs (Fig. 6).

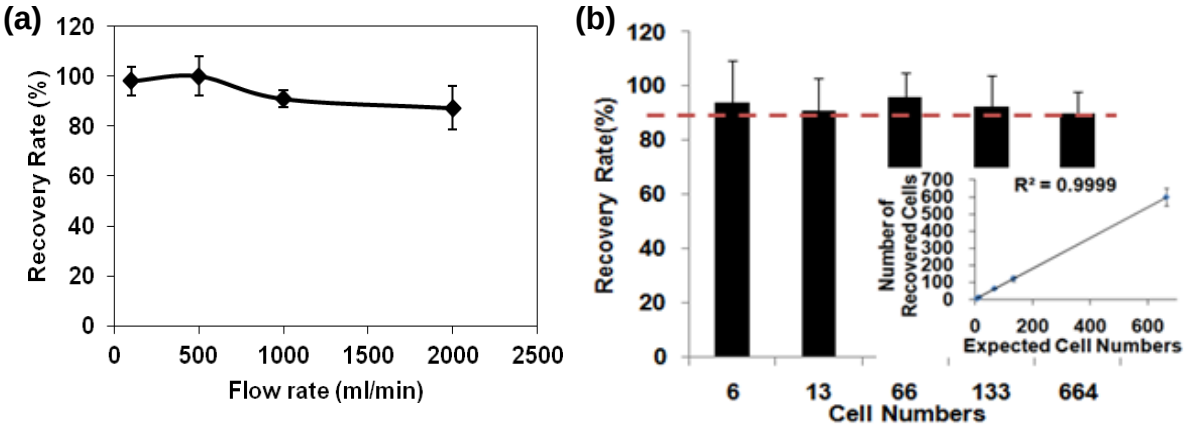

Figure 5. (a) Recovery rate as a function of flow rate (b) Recovery rate vs. input cell number

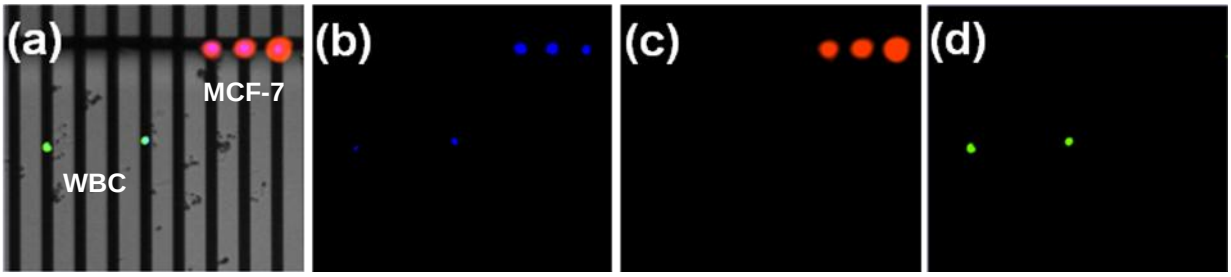

Figure 6. Staining images after isolation process of rare cells (a) Merged image (b) DAPI (c) Cytokeratin (d) CD45

CONCLUSION

We demonstrated that the novel 3D-flow filter can capture rare cells in whole blood with high recovery yield (over 90%) and throughput (up to 1000 ml min^{-1}). With the enhanced capture yield and throughput, the proposed device can be used as an efficient rare-cell-analyzing tool for blood-based diagnostics.

REFERENCES

[1] M. Cristofanilli, et al., *New England Journal of Medicine*, 351, 781-791 (2004).
[2] S. Damani, et al., *Science Translational Medicine*, 126, 126ra33 (2012).
[3] P. Mehlen, et al., *Nature Reviews Cancer*, 6, 449 (2006).
[4] H. Mohamed, et al., *Journal of Chromatogrphy A*, 1216, 8289 (2009).
[5] S. Tan, et al., *Biosensors and Bioelectronics*, 26, 1701 (2010).

CONTACT

June-Young Lee, tel: +82-31-280-1821, iamjy.lee@samsung.com