A MICROMIXER FOR CONTINUOUS LABELING OF CIRCULATING TUMOR CELLS WITH MICRO-BEADS AS A HIGHLY SELECTIVE ISOLATION

MingXian Lin[1], Kyung-A Hyun[1], Hui-Sung Moon[2], Tae Seok Sim[2], Jeong-Gun Lee[2], Jae Chan Park[2], Hyo-Il Jung[1]

[1]Yonsei University, Republic of Korea, [2]Samsung Advanced Institute of Technology, Republic of Korea

ABSTRACT

Rare circulating tumor cells (CTCs) have been identified in peripheral blood from cancer patients and have been proved to be a main cause of metastatic disease. Current strategies for size based on isolation of CTCs has been technically challenged owing to their overlapped size value(e.g., CTCs ~16-20μm diameter and leukocytes ~8-14 μm). Here we describe the development of a unique microfluidic device, Taylor Gortler(TG) mixer, capable of efficient and selective separation of viable CTCs from peripheral whole blood samples, operated by the interaction of target CTCs with antibody (EpCAM)-coated microbeads under microfluidic channel, as a result the size of the CTCs will be increased. First of all, we used red-green fluorescence beads to evaluate the mixing efficiency of the device. And then, we have gotten binding efficiency by using breast cancer cell and anti-epithelial cell adhesion molecule(EpCAM) coated microbeads through TG mixer.

KEYWORDS

Circulating Tumor cells, micromixer, EpCAM antibody, microbeads, microfilter

INTRODUCTION

Because of its potentials and advantages such as less time consumption, small amounts of sample and reagent, lower cost and high throughput the microfluidic devices have been used widely in chemical and biological fields and also developed rapidly over the past decade. Although highly successful, an important issue is being focused on overcoming a number of performance limitations, one of which is low mixing efficiency. The difficulty in rapidly mixing reagents results from the fact that the system is often restricted to the laminar flow regime (Reynolds number, $Re < 2000$, the critical value for turbulent flow) and also because the channel sizes are too small (typically <100μm) to operate conventional mixing mechanisms[1]. Therefore, effective mixing on micro scale has become a challenging problem to many of the microfluidic systems. Recently, a lot of researchers have proposed passive micromixers which are designed with special microstructures for improvement of mixing in microfluidic devices to induce fast mixing between fluid streams such as chaotic mixer [2]. We also found that several Taylor Gortler vortices perpendicular to the main flow direction was observed in a curved rectangular section channel. We fabricated Taylor Gortler(TG) micromixer having a high aspect ratio. It focuses on improvement of mixing efficiency between breast cancer cells and microbeads by understanding the effects of different flow rates, diffusion coefficient and geometric parameters such as channel angle.

EXPERIMENT

Fig.1 shows TG micromixer device and the operation mechanism for the breast cancer cell tagged with EpCAM coated microbead. TG mixer consists of sharp-edged turns, rectangular cross section and zigzag microchannel integrating a "T" inlet junction. In the microfluidic channels, secondary flows as a kind of Taylor Gortler vortex are formed due to the centrifugal instability caused by the shape of the corrugated channel. It makes several counter-rotating secondary vortices [3].

The silicon mold of the microchip was fabricated by using deep reactive ion etching process. PDMS (Sylgard 184, Dow Corning) was cast on top of the mold and degassed, after passing baked, separated from the mold, punched inlet and out let, bonded to a slide glass through plasma bonding. TG mixer has 80μm width and 400μm high; angles of the channel are 110° and 130°, respectively.

Microbead (3μm) is chemically functionalized with anti-epithelial cell adhesion molecule (EpCAM). Anti-EpCAM provides specific binding between breast cancer cell and microbead through Taylor Gortler vortices.

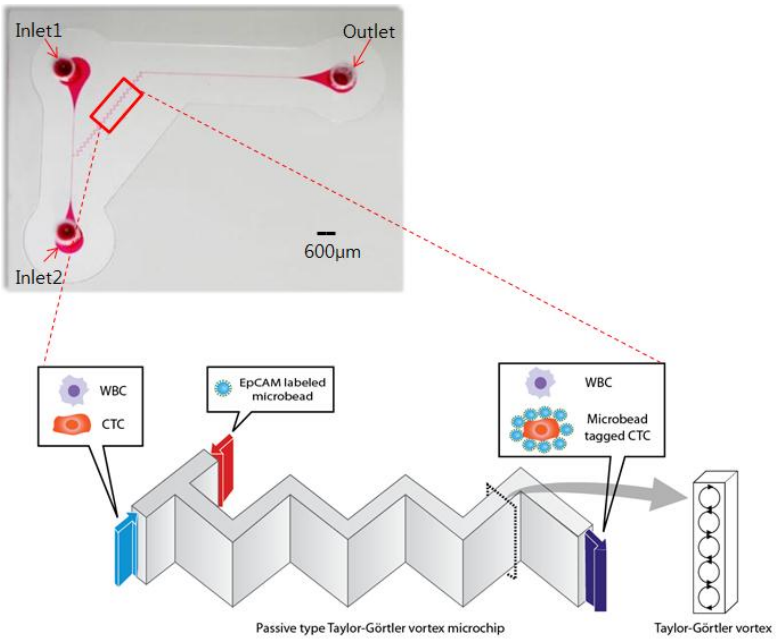

Figure 1 A photograph and schematic diagram of wavy duct for tagging circulating tumor cell (CTC) with anti-EpCAM coated microbeads.

RESULTS AND DISCUSSION

Fig 2 shows the red-green color composite images captured from CCD camera using 522 and 603nm fluorescence emission wavelengths. At low flow rates, i.e., 100µl/min, the image exhibits sharp red–green bands with a clear fluid interface in the 4th microchannel, which indicates little mixing occurred in this condition. With the increase of the flow rate in both of the channel, fluid interfaces become uncertain and broadened. As a result, when the flow rate has been 600µl/min, the fluorescence intensities are observed more uniformly and had yellow streamlines as expected for a mixture of two fluorescent species.

Fig. 3 presents the binding experiments of breast cancer cells and EpCAM coated microbeads to determine the best condition for microbead labeling. Concentrations of breast cancer cell and the EpCAM coated microbead are 2 x 105/ml and 2 x 106/ml, respectively. The sample was passed through 110° and 130° of the mixer at each flow rate of 300µl/min and 600µl/min. Based on the outlet mixture as shown in Fig 5, we propose that the high velocity and low angle of the channel increase the binding efficiency. In addition the recovery of the breast cancer cell is more than 70%.

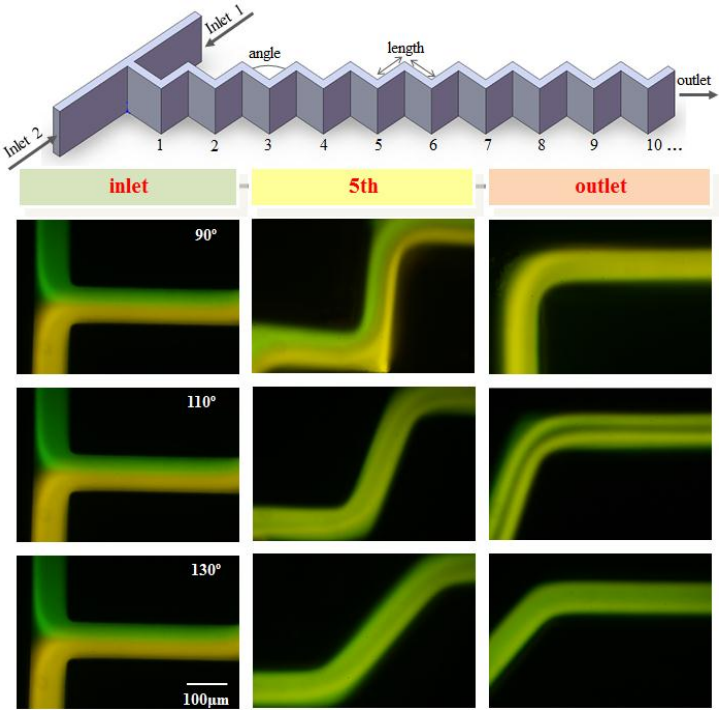

Figure 2 Mixing image of fluorescent beads in 600µl/min and the channel angle of 90°, 110°, 130°, respectively.

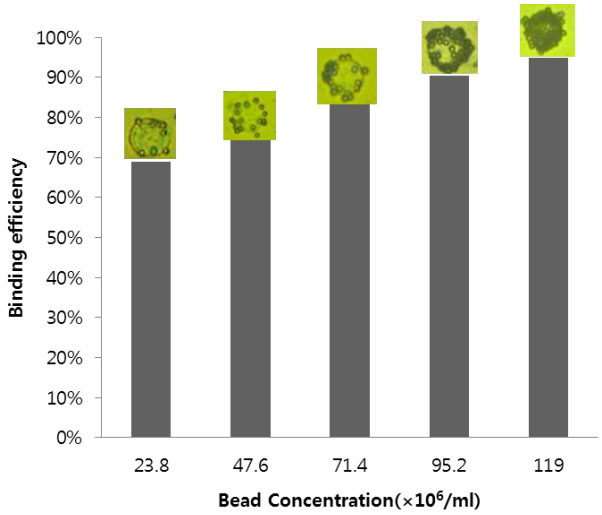

Figure 3 Binding efficiency of the microbeads with MCF-7 cells.

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CONTACT

Hyo-Il Jung +82-2-2123-5814 or uridle7@yonsei.ac.kr