Seamless Multi-fluorescence Labeling in a Microfluidic Disk via Deterministic Vent Valves

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ABSTRACT

For cellular diagnostic applications, multi-fluorescence labeling is often required. Traditionally, the protocol of repeatedly incubation, washing and removal liquid, is manual and tedious. We present a new microfluidic device with vent valves to allow precise timing in liquid delivery and demonstrated the multi-fluorescent labeling process on the disk via the operation of a vent control plate (VCP). The multi-fluorescent labeling process is repeated by sequential operation of the VCP via fixed interval rotation. The operation of the vent valve is simple and fast. Multi-fluorescence labeling processes are easy to carry out on the disk. To prove of concept, MCF7 labeled with magnetic beads were trapped on the disk first and we performed anti CK-FITC and Hoechst 33342 staining seamlessly on-disk. The valve and the disk would be applicable to rare cell detection, such as CTC detection.

KEYWORDS

Disk, fluoresscence labeling, vent valve.

INTRODUCTION

Detection and quantification of target cells in blood is important clinically. For example, the quantity of circulating tumor cells (CTCs) in the peripheral blood might be useful to predict metastasis, forecast disease stage, and monitor the response of adjuvant therapy[1]. For enumeration of target cells, multiple labeling processes were often required for analysis in cellular studies. Traditionally, the protocol of repeatedly incubation, washing and removal liquid on a tube is manual and tedious. Disk-based design constitutes another class of microfluidic device for biomedical studies which leverages the centrifugal force directly for fluid handling and requires no external interconnects to induce fluid movement. This allows the compact fluidic network to be contained within a single disk[2,3]. Excellent reviews on their recent development for DNA assay and immunological protein studies have been published[2]. However, most of valves on disk are passive valves, such as capillary valve or hydrophobic valve. The fabrications of these disks are complex due to require ultra-precise structures or local surface modifications. Beside, the operation results depend on fluid characteristics, surface properties and channels dimensions. This work presents an innovative active vent valve to enable easy to use and fabricate. We also demonstrated a centrifugal microfluidic disk to perform fluorescence labeling of cells seamlessly on-disk via vent valves and a vent control plate (VCP), designed to allow precise timing of liquid delivery during the multiple label/wash processes.

EXPERIMENT

The physical principle of vent valves to control liquid delivery is shown in Fig. 1 (a). A vent control plate (VCP) is used to operate vents to open or close on the disk. Fig. 1 (b) shows the vent valve is opened just when the VCP hole is aligned in this vent, and the other valves are still closed. The sequential transfer of liquid from one reservoir to another is performed by adjusting the rotational angle of the VCP. The vent valve is active valve, and the advantages of the proposed valve include simple to operate and easy to fabricate.

Fig. 2 illustrates the microfluidic processes of enriching target cells and labeling fluorescence. Target cells labeled with immuno-magnetic beads and loaded into the disk. After disk spinning, the magnetically-labeled target cells are trapped in the capturing reservoir by magnets, as shown in Fig 2 (a). After target cells captured, the steps of fluorescent staining are executed. The steps include: incubation (Fig 2 (b)) and washing (Fig 2 (c)). To stain fluorescence, opening Valve 1 and spinning disk, the fluorescent antibody are moved from Reagent Reservoir 1 to the capturing reservoir and held here since the amount of liquid is not enough to pass the crest point of the siphon structure. To wash cells, opening Valve 2 and spinning disk, the buffer in Reagent Reservoir 2 is pushed to the capturing reservoir and propels the prior liquid over the crest point of the siphon structure. With this difference in radial position, the liquid in the capturing reservoir is removed. The multi-fluorescent labeling process is repeated by sequential operation of the VCP via fixed interval rotation.

Fig. 3 shows the photos of multi-fluorescence labeling process. Blue and red buffer were loaded into the reagent reservoirs and the VCP was put on the disk (Fig 3(a)). Fig 3 (b)-(g) show the processes for keeping and removing buffer in the reservoir are repeatedly by sequential operation of the VCP. The method could reduce complex sample transfer and manual operation. To compare with perfusion system, large reagent consumption could be avoided.

Fig. 4 shows the performance with fluorescent labeling on target cells. MCF7 cells were labeled by anti-EpCAM-PE and anti-PE magnetic beads and then trapped on the top surface of the capturing reservoir (Fig 4(a)). Anti CK-FITC and Hoechst 33342 was used to label MCF7. Fig 4(b) and (e) showed the results. The cell loss after fluorescent staining is around 5~10%. The valve and the disk would be applicable to rare cell detection, such as CTC detection.

CONCLUSION

The disk device presents multi-fluorescence labeling processes on disk. The total process decreases multiple manual preparatory steps. This disk would also be applicable to many rare cell detection scenarios with distinct immunological features, such as stem cell detection and prenatal diagnosis.

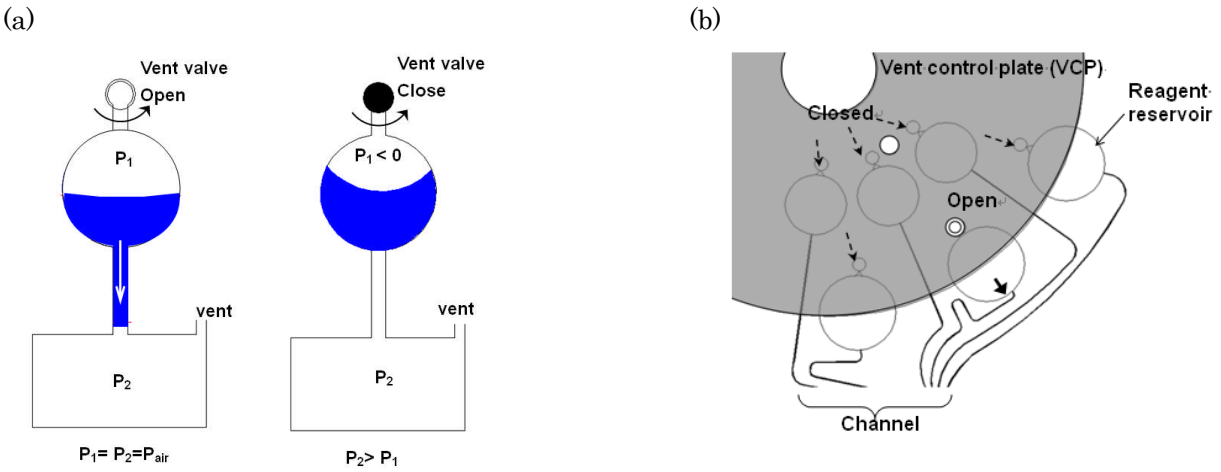

Figure 1: Schematic diagram of vent valve's principle and operation. (a) When the vent is closed, the generated air counter-pressure reachs the equilibrium with the centrifugally generated pressure (ΔPc) in the liquid, effectively stopping. In constrant, the liquid could flow into outer reservoir by centrifugally generated pumping pressure while the vent is open. (b) A vent control plate (VCP) is applied to operate vents to open or close.

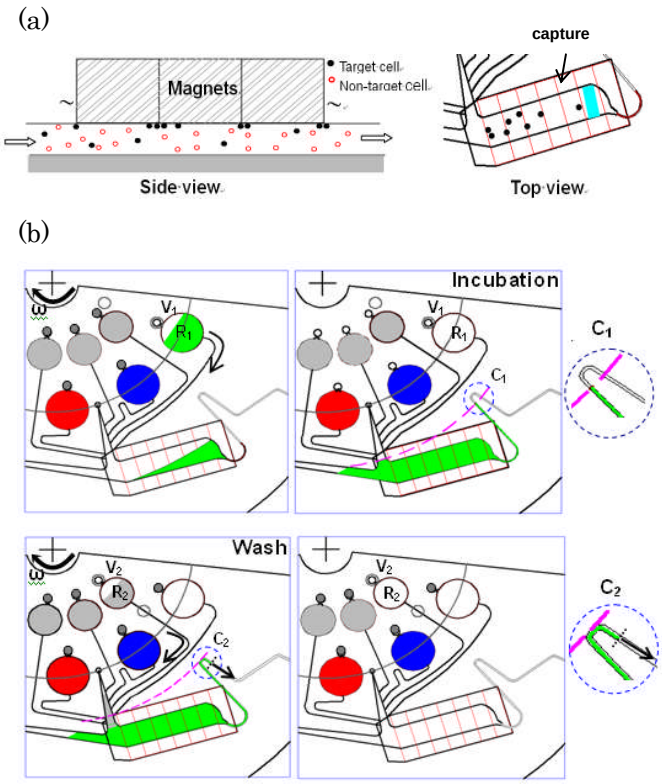

Figure 2. (a) The magnetically-labeled target cells are trapped on the top surface of the capturing reservoir by the magnet array when flow passes across. (b) A batch process is used to stain fluorescent antibodies. The steps include: Incubation and washing. A siphon structure combined with air vent valve are designed to hold and remove liquid in capture reservoir. The fluorescent labeling process is repeated by sequential operation of the VCP via fixed interval rotation.

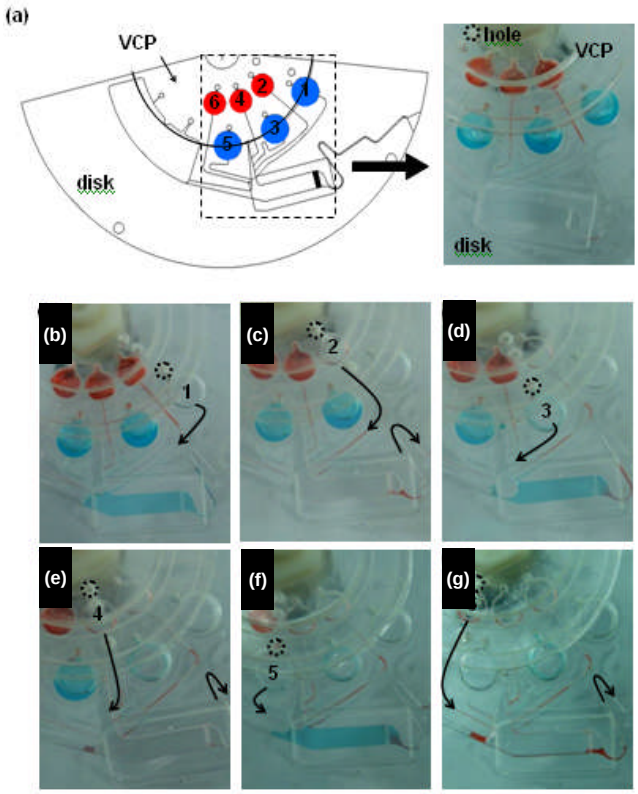

Figure 3. Photo of multi-fluorescence labeling process.

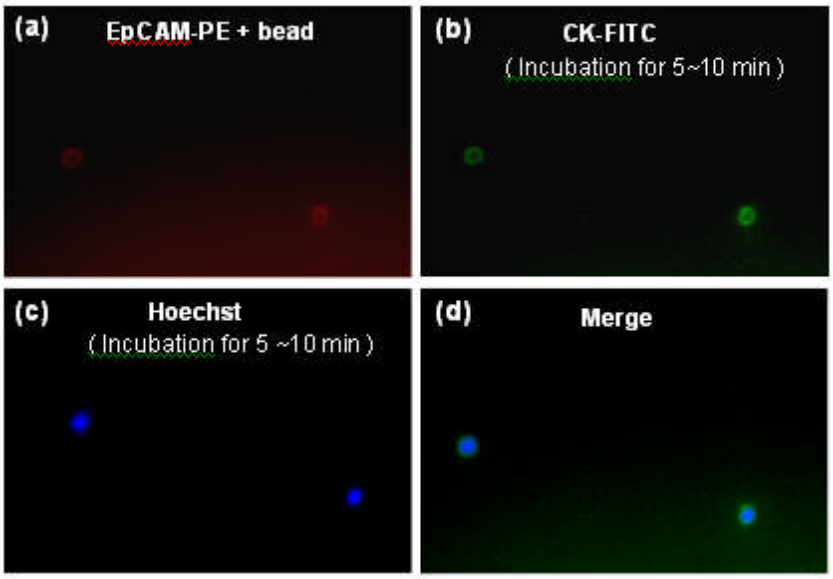

Figure 4 Fluorescence images of captured MCF7 cells stained with anti-CK-FITC and Hoechst 33342 on the disk. (a) MCF7 cells were stained by anti-EpCAM-PE and anti-PE magnetic beads and captured on the disk. (b) and (c) These two images show MCF7 cells labeled with anti-CK-FITC and Hoechst 33342 after performing the multi-fluorescence labeling process, and (d) Merged image from (b) and (d).

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