One-step White Blood Cell Separation from Whole Blood On a Centrifugal Microfluidic Device

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ABSTRACT

In this work, we propose a fully automated WBCs isolation device from biological sample utilizing centrifugal microfluidics on a polymer based CD platform. Using the novel Laser Irradiated Ferrowax Microvalves (LIFM) and liquid density gradient medium on CD platform, the total process of a blood sample loading on the density gradient medium, fractionating to concentrate the WBC, decanting plasma layer, and WBC isolation was finished within 5 minutes with only manual step of adding diluted 100 μL of whole blood. The hematocytometry results showed that the number of WBC was as good as the sample prepared in conventional manual method. Furthermore, As a CD could prepare maximum 24 samples the automatic method is more useful for the high throughput cell isolation as well as enables one to enhance reproducibility, laborious and time-consuming process for the RNA purification.

Keywords: White Blood Cells (WBC), Microvalve, Centrifugal Microfluidics

1 INTRODUCTION

The isolation of intact, high quality RNA is critical to successful gene expression analysis experiments including RT-PCR, RNA mapping, in vitro translation, Q-RT-PCR, RNA labeling, and cDNA library generation. Automation of RNA purification enhances reproducibility and saves time and labor.

For practical reasons, whole blood is often fractionated to concentrate the nucleated cells (i.e., the WBCs) prior to RNA extraction or immunoselection. Removing plasma and red cells eliminates many of the nucleases and inhibitors from blood and reduces the sample volume by at least ten-fold, which allows RNA extraction to be carried out in microfuge tubes (< 2 ml) instead of larger vessels. Also, most protocols for immunoselection of leukocyte subsets are more efficient and cost-effective when carried out on fractionated leukocytes rather than whole blood. Density gradient centrifugation is probably the most common method for fractionating whole blood [1]. Although this commercial available method is very useful in vitro assays, it has many shortcomings because process has many manual steps as well as often time-consuming process. First, blood manually carefully layered onto the medium. Second, isolated WBC is harvested by very carefully pipetting them from the liquid interface after centrifugation. As a result, manual process become reasons of error of the cell separation yield.

We have demonstrated an innovative Laser Irradiated Ferrowax Microvalves (LIFM) that is based on phase transition of ferrowax, paraffin wax embedded with 10 nm sized ferrooxide nanoparticles. Compared to the conventional phase change based microvalves, the control of multiple microvalves was simple by using single laser diode instead of multiple embedded microheaters.15, 36 Furthermore, LIFM is not very sensitive to rotation speed or surface properties. Both Normally Closed (NC)–LIFM and Normally Opened (NO)–LIFM were demonstrated and various fluidic functions such as valving, metering, mixing, and distribution were demonstrated using centrifugal microfluidic pumping. In addition, the response time to open the channel by melting the wax was dramatically reduced from 2 ~ 10 sec. to less than 0.5 sec. because the laser beam effectively heat the nanoparticles embedded in the paraffin wax matrix [2, 3].

Here, we propose a fully automated WBCs extraction device from biological sample utilizing centrifugal microfluidics on a polymer based CD platform. Using the innovative Laser Irradiated Ferrowax Microvalves (LIFM) together with cell separation method using liquid density gradient medium (DGM), we could, for the first time, demonstrate a fully automated WBC separation from whole blood on a CD.

2 MATERIALS AND METHOD

As shown in Fig. 1A, schematic illustration of operation of Laser Irradiated Ferrowax Microvalves (LIFM) on a CD. By adjusting the position of laser beam irradiation, the main channel could be opened for centrifugal pumping.

As shown in Fig. 1B, the polycarbonate (PC) CD is composed of a top layer consisting of various inlet holes and a bottom layer consisting of channels and chambers. The channel width was 1 mm and the depth was 100 μm. The depth of the chamber was 3 mm. The inlet holes and channels were produced by conventional CNC (computer-controlled machine), and the two separate layers were bonded with a double sided adhesive tape. The microfluidic layouts designed by using CAD (Computer-Aided Design) were cut by using a computer controlled vinyl film cutter.

The ferrowax valve is made of a nanocomposite materials composed of 50 % of paraffin wax and 50 % ferrofluids; 10 nm sized ferrooxide nanoparticles dispersed

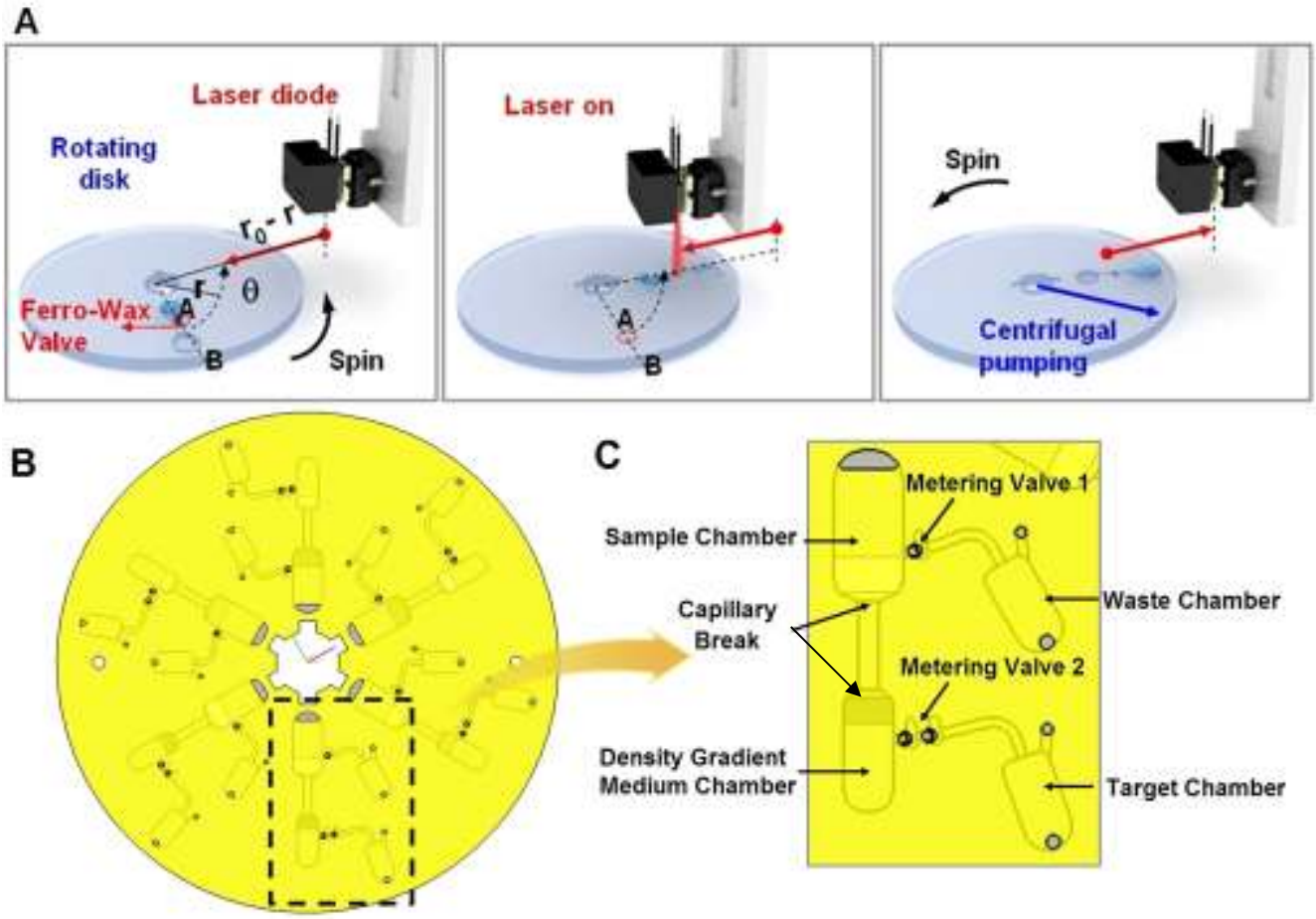

Fig. 1 (A) Schematic diagram of the ferrowax valve operation on a CD. In order to transfer liquid from reservoir A to reservoir B, the CD is rotated with the angle θ to the laser home and the laser diode is moved to the valve position with the distance ($r-r_0$). Then, laser with power of 1.5 W was applied for 1 sec to melt the ferrowax and the disk is spin to pump liquid from reservoir A to reservoir B. **(B)** Schematic diagram of the microfluidic layout on a disc**. (C)** Detailed description and function of the WBC separation microfluidics layout on a disc.

in oil. The detail procedure of the fabrication and characterization of LIFM is previously reported.35

Fractionation of whole blood sample was done by using the liquid density gradient medium (Osmolarity: ± 15 mOsm, LymphoprepTM, Oslo, Norway)

In order to measure number of the isolated WBC, we used hematocytometry.

3 RESULTS AND DISCUSSION

As shown in Fig. 1, a microfluidic layout was designed to fully integrate the WBC separation from whole blood on a CD. Table 1 shows the spin program at each operation step. The images shown in Fig. 2 were obtained during the rotation using the CCD camera and strobe light.

As shown in Fig. 1B ~ 1C. First, 100 μL of whole blood and density gradient medium (DGM) were added to the sample chamber and DGM chamber, respectively, as shown in Fig. 2A. Capillary break region (depth of 50 μm, width of 2 mm) was designed so that sample was not mixed with DGM. In order to separate WBC from the mixture with whole blood and DGM, the CD was spun with an acceleration of 30 Hz s-1 and a maximum speed of 60 Hz s -1 for 300 s (Fig. 2B ~ D). For the purification of the concentrated WBC, at first, metering valve 1 is opened by laser irradiation and waste solution was transferred to waste chamber by centrifugal pumping (Fig. 2E). Then, isolated WBC solution positioned between metering valve 1 and 2 was transferred to target sample chamber with same manner as described above (Fig. 2F). Fig. 2G ~ I show that the WBC separation process was good working. The separated WBC was counted by hematocytometry. The yield of WBC was 28700 +/- 1700 per 100 μL whole blood, which was 54% of the manual preparation (51363 +/- 9513/100 μL whole blood). In case of preparation-to-preparation variation, the automatic isolation showed only 6% of CV. In addition, comparing to the time consuming manual cell

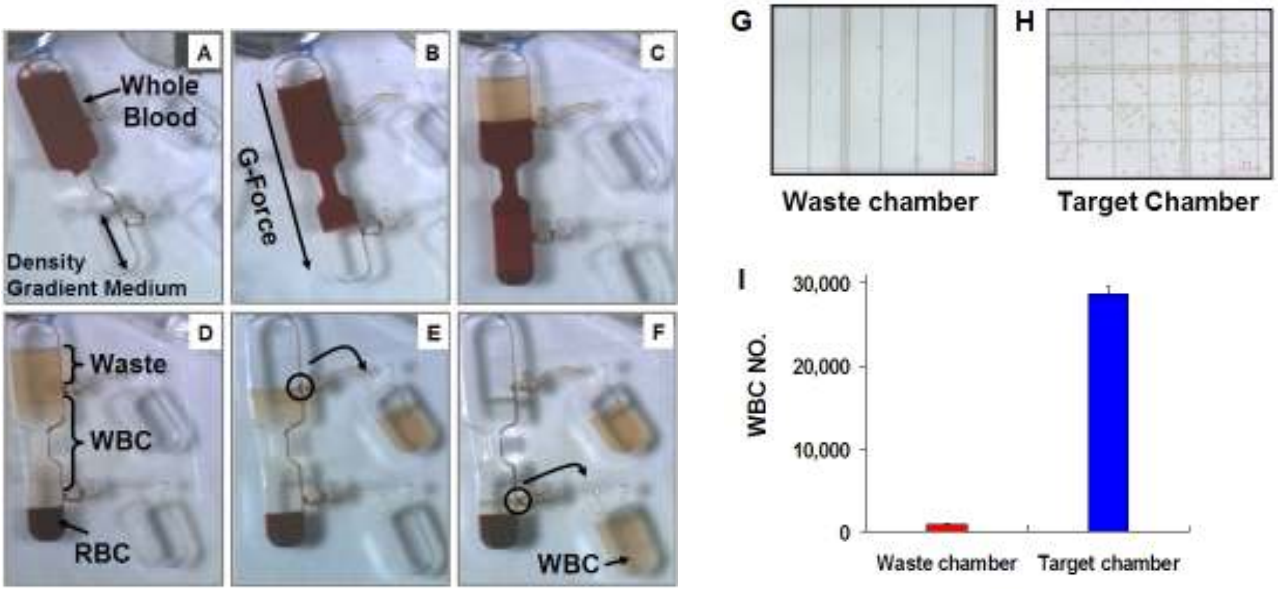

Fig. 2 Photo images captured during the operation of automated WBC separation of the spin and resulting data. **(A ~ F)** Photo images captured during the operation of the spin. **(G ~H)** Microscope view images of the each chamber after completed WBC separation. **(I)** Hematocytometry results from WBC separation by the fully automated method on a CD.

preparation (one hour for three samples), the automatic one took just 5 minutes/CD.

Table 1. A spin program for the microfluidics on a CD

Spin No.	Spin speed (Hz)	Time (sec)	Operation
	-	-	Preload DGM 100 μL to DGM chamber
1	-	-	Input whole blood of 100 μL to sample chamber
2	60	300	Fractionating whole blood
3	30	5	Metering valve 1 opened, transfer plasma decant to waste chamber
4	30	5	Metering valve 2 opened, transfer WBC to WBC chamber

4 CONCLUSION

By combining with the novel LIFM based centrifugal microfluidics platform and a liquid density gradient materials, we could, for the first time, a fully automated WBC isolation platform is developed.

Consequently, automated intact WBC isolation platform enables one to enhance reproducibility and saves time and labour for the RNA purification.

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