CONTINUOUS-FLOW MICROFLUIDIC BLOOD CELL SORTING FOR UNPROCESSED WHOLE BLOOD USING SURFACE-MICROMACHINED MICROFILTRATION MEMBRANES

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ABSTRACT

We report a microfluidic chip for continuous-flow isolation and sorting of WBCs from whole blood with high throughput and separation efficiency. The microfluidic cell sorting chip leveraged the crossflow filtration scheme in conjunction with a surface-micromachined poly(dimethylsiloxane) (PDMS) microfiltration membrane (PMM) with high porosity. With a sample throughput of 1 mL hr^{-1}, the microfluidic cell sorting chip could recover 27.4 ± 4.9% WBCs with a purity of 93.5 ± 0.5%. The microfluidic cell sorting chip holds promise as an upstream component for blood sample preparation and analysis in integrated blood-on-a-chip systems.

KEYWORDS: Blood Cell, Separation, Membrane, Microfluidics

INTRODUCTION

White blood cells (WBCs) constitute about 0.1% of the blood cells, yet they play a critical role in innate and adaptive immune responses against pathogenic infections, allergic conditions, and malignancies and thus contain rich information about the immune status of the body. Rapid isolation of WBCs directly from whole blood is a prerequisite for any integrated immunoassay platform designed for examining WBC phenotypes and functions. However, such functionality is still challenging for blood-on-chip systems, as existing microfluidic cell sorting techniques are incapable of efficiently processing unprocessed whole blood on chip with concurrent high throughput and cell purity [1,2].

RESULTS AND DISCUSSION

Herein we report a microfluidic chip for continuous-flow isolation and sorting of WBCs from whole blood with high throughput and separation efficiency. The microfluidic cell sorting chip leveraged the crossflow filtration scheme in conjunction with a high-porosity poly(dimethylsiloxane) (PDMS)

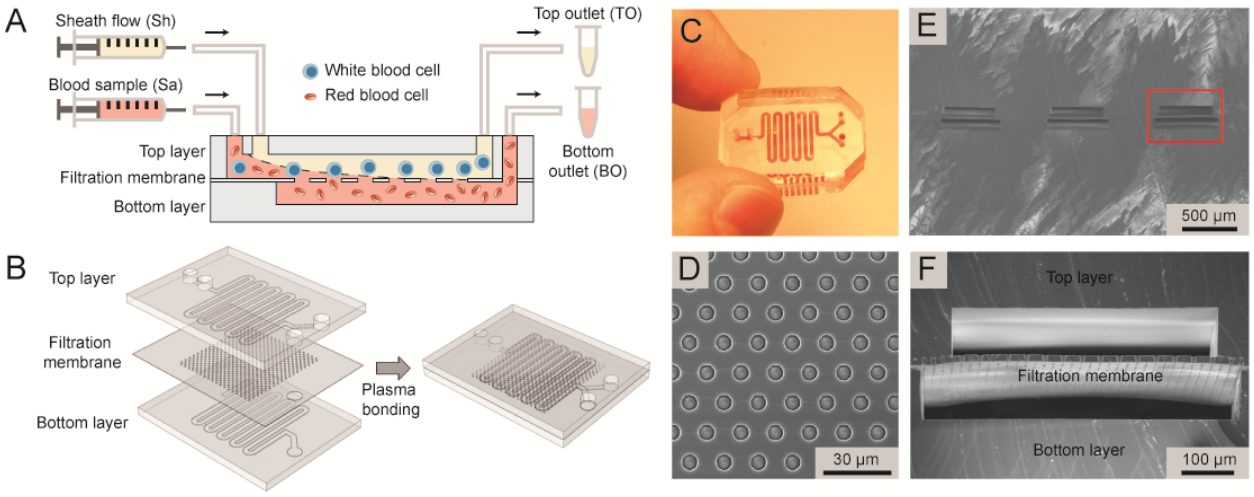

Figure 1: Continuous-flow microfluidic cell sorting using crossflow filtration with PDMS microfiltration membranes (PMMs).

microfiltration membrane (PMM) (Fig. 1A&B). Wafer-scale fabrication of 1 cm × 1 cm PMMs with 4 μm pores and 10% porosity was achieved using our novel surface micromachining strategy [3] (Fig. 1C&D). Standard soft lithography was used to generated the top and bottom microfluidic channels for the microfluidic cell sorting chip. The microfluidic chip realizes separation by using a crossflow that is perpendicular to the PMM to push undesired red blood cells (RBCs) to pass across the PMM and a continuous tangential main flow to carry away WBCs.

We validated the separation performance of the microfluidic cell sorting chip using two different sized microbeads with diameters of 3 μm (yellow) and 11 μm (blue), respectively. The sample flow contained the two types of microbeads both with a concentration of 1×10^6 mL^{-1}. Fluorescent microscopic images illustrated the separation of microbeads under v_{sample} = 2 mL hr^{-1} and v_{sheath} = 4 mL hr^{-1} (Fig. 2A). Before separation, the microbead mixture contained an equal amount of 3 μm (yellow) and 11 μm (blue) beads. After separation, 97.3 ± 0.5% of 3 μm microbeads were removed from the solution collected at the top outlet, whereas 96.9 ± 0.4% of 11 μm microbeads remained. We further examined the effect of v_{sample} and v_{sheath} on the separation of microbeads, and found that the flow rate ratio, v_{sample} / v_{sheath}, rather than the magnitude of the flow rate, mainly determined the separation efficiency (Fig. 2B&C).

We further applied the microfluidic cell sorting chip for separation of WBCs from unprocessed porcine whole blood. Blood cells (including both WBCs and RBCs) collected from the top outlet were examined and counted under phase contrast microscopy. WBCs were identified by their relatively larger size compared to RBCs (Fig. 3A). With a sample throughput of 1 mL hr^{-1}, which was 270 times greater than those demonstrated previously using microfluidic filtration devices [2], the microfluidic cell sorting chip could recover 27.4 ± 4.9% WBCs with a purity of 93.5 ± 0.5% (Fig. 3B&C). We conducted cell viability assays for WBCs before and after separation, with data showing that microfluidic cell sorting had no significant effect on WBC viability (Fig. 3D).

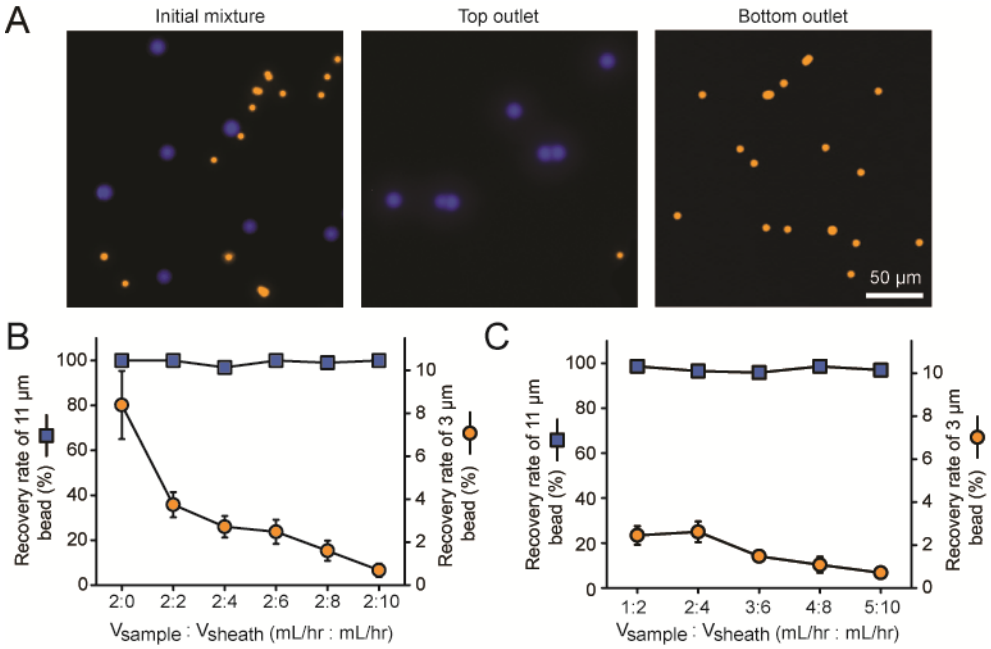

Figure 2: Characterization of separation performance using different sized microbeads. (A) Representative fluorescent microscopic images before and after separation. 11 μm microbeads were in blue and 3 μm ones were in yellow. (B) Recovery rates of microbeads at the top outlet, with v_{sample} = 2 mL hr^{-1} and v_{sheath} from 0 to 10 mL hr^{-1}. (C) Recovery rates of microbeads at the top outlet, with the sample and sheath flow rates proportionally increasing from 1 mL hr^{-1} : 2 mL hr^{-1} to 5 mL hr^{-1} : 10 mL hr^{-1}.

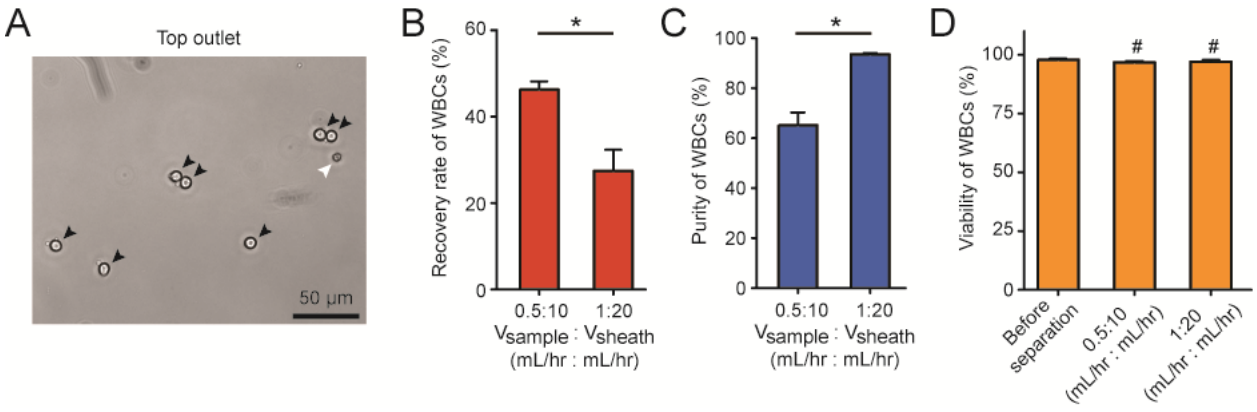

Figure 3: Separation of WBCs from unprocessed whole blood. (A) Representative bright-field image showing blood cells after separation. WBCs (black arrow head) were identified by their relatively larger size compared to RBCs (white arrow head). (B&C) Recovery rate and purity of WBCs collected at the top outlet as a function of v_{sample} and v_{sheath}. (D) Viability of WBCs before and after separation.

CONCLUSION

We demonstrated a microfluidic cell sorting chip that could achieve continuous-flow separation of WBCs from unprocessed whole blood with high throughput and cell purity. We carefully validated separation performance of the chip under different flow conditions and using different samples, including microbead mixtures and unprocessed whole blood. When processing unprocessed whole blood, the microfluidic cell sorting chip recovered 27.4 ± 4.9% WBCs with a purity as high as 93.5 ± 0.5% at a sample throughput of 1 mL hr^{-1}. With all these highly desirable features, we envision that the microfluidic cell sorting chip demonstrated in this work can serve as a standard cell sorting component for upstream sample preparation in future highly integrated blood-on-a-chip systems.

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