ACOUSTIC DEVICE FOR SELECTIVE PLATELET EXTRACTION FROM WHOLE BLOOD

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ABSTRACT

Platelet separation from blood is fundamental technique for biochemical analyses. In this article, we proposed a method to collect platelets from whole blood utilizing standing surface acoustic waves (SSAWs) in a microfluidic device. A polydimethylsiloxane (PDMS) microfluidic device was integrated with interdigitated transducers (IDTs) on a piezoelectric substrate. Whole blood sample was hydrodynamically focused by sheath flow from side inlets. Pressure nodes were designed to locate at side walls, thereby collecting platelets with minimum shear effect. Finally, the RBC clearance ratio from whole blood was found to be 99% and the purity of platelets was close to 98%.

KEYWORDS: Platelets, Separation, Standing surface acoustic wave, Microfluidics

INTRODUCTION

Recently, platelet separation from whole blood have gained much attention for biological researches and clinical applications. Platelets have been found to be involved in not only haemostasis and thrombosis, but also cell regeneration¹, angiogenesis², tumor metastasis³, etc. Conventional centrifugation method is widely used for platelet separation, yet it may lead to platelet activation that is caused by mechanical shear stress during high-speed centrifugation. In fact, it is difficult to separate non-activated platelets from whole blood due to high shear-sensitivity of platelets⁴.

Various techniques have been proposed by utilizing dielectric⁵, hydrophoretic⁶, and acoustic⁷,⁸ forces for the continuous separation of blood cells. Recently, acoustic devices have contributed to advances in particle manipulation, which is highly suitable for treating bioparticles, because it is label-free and does not require pre-treatment of the particles⁹. Tony et al. demonstrated a novel method to separate poly-dispersed particle suspensions in microfluidic channel using standing surface acoustic waves (SSAWs)⁷.

In this study, we propose a novel application of SSAW techniques in microfluidic channel for the separation of platelets from whole blood. The objectives of the study are to achieve separation of high purity of platelets from undiluted whole blood. Additionally, another advantage of the current design is to separate platelets without activation which is used to occur in conventional centrifugal methods. Considering these design requirements, IDTs and microchannel have been carefully designed, and the hydrodynamic focusing technique also has been adopted.

EXPERIMENTAL

Figure 1 describes a schematic of our device. A polydimethylsiloxane (PDMS) microfluidic channel with two inlets and two outlets was fabricated and integrated with a couple of IDT electrode patterned on a piezoelectric substrate. The channel was 150 μm in width and 50 μm in depth, and the width and the pitch of the IDT were 125 μm and 500 μm, respectively.

Figure 1: Selective platelet separation from whole blood in the acoustic device
Here, the working wavelength ($\lambda$) was determined by the relation, $f = c / \lambda^{10}$, where $c$ is the SAW velocity of the LiNbO$_3$ substrate and $f$ is the applied signal frequency set at 7.54 MHz.

Blood was obtained from healthy volunteers, who were not on any medication and who provided informed consent. Venous blood samples were drawn from the antecubital vein and collected in vacutainers (6ml, BD, Franklin Lakes, NJ) that contained the anticoagulant, EDTA(K$_2$). It is worth noting that whole blood was directly used without any pre-treatment. As shown in Fig. 1b, each blood sample was injected into the central inlet, while sheath flow was injected from two side inlets by a Hamilton glass syringe (5.0ml, 1005TTL SYR, Hamilton), which was pushed by a syringe pump (KDS101, KDScientific).

To prevent shear-induced activation of platelets, the blood sample flow was hydrodynamically focused by sheath flow. When the blood particles are subject to the acoustical forces of SSAsWs, RBCs and WBCs were pushed to the side walls, whereas platelets kept flowing in the midstream due to the difference in size. Since the primary acoustic force ($F_r \propto V_p \propto r_p^3$) is much greater than the viscous force ($F_\nu \propto r_p$), larger particles experienced a greater net force in SSAW field and this attributes to the selective collection of platelets and removed RBCs and WBCs at each outlet. For a quantitative analysis of the separated samples, each collected sample from three outlets was analyzed by a flow cytometer (FACScalibur, BD Bioscience, CA).

RESULTS AND DISCUSSION

Figure 2 shows the result of calculated RBC clearance ratio with various applied voltages ranging from 17V to 19V. Here the RBC clearance ratio was defined as the ratio of the number of RBCs found at outlet B to the total number of injected RBCs. The RBC clearance ratio was over 99% in the region between 18V and 19V. From this we could evaluate how effectively the separation device works and select the optimal input voltage of 18.5V. The flow rates of the sample and sheath flow were 0.25μL/min and 5μL/min, respectively.

Under the same condition, we conducted platelet separation and the result is presented in Figure 3. To estimate the purity of the separated platelets, cytometric analysis was implemented. At outlet A, RBCs were successfully removed from the whole blood sample while a large number of platelets were collected. Almost all the unwanted RBCs were removed from the injected sample and enriched at outlet B. Here the purity is defined as the ratio of the number of platelets to the total number of particles found at outlet A. By counting 10,000 events with the prescribed gates, the distribution of the cell population could be obtained. This resulted in the RBC clearance ratio of over 99% and the purity of platelets close to 98%, which was higher than previous results (85%$^6$ and 95%$^5$).

![Figure 2. (a) The RBC clearance ratio as a function of the input voltage. (b-d) Microscopic images at applied voltages of less than 17V, 17V, and 18.5V, respectively.](image)

CONCLUSION

Conclusively, our device provides high efficiency of separation and availability of the direct use of whole blood. Since the present technique also enables easy operating alteration, one can easily apply this technique for the extraction of platelet-rich-plasma, platelet-poor-plasma, or platelet-derived microparticles.
Figure 3. Flow cytometric scattergrams. (a) Whole blood sample before separation. (b) The recovery ratio at each outlet for the collected sample after separation. (c-d) The distributions of blood particles at outlets A and B after separation.

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REFERENCES

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