PLATELET ENRICHMENT IN A CONTINUOUS AND CLOG-FREE MICROFLUIDIC FILTER WITH SUNFLOWER HEAD GEOMETRY

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ABSTRACT

Size-based separation of platelets (1-3µm) from a background of RBCs (~7µm) in microfluidic devices is extremely challenging as the RBCs are highly deformable and can pass through gaps as small as 3µm. High shear stresses during sorting can activate platelets causing aggregation. We report a novel radial pillar device design that can enrich the platelet population by >25-fold. Our device can work with whole or diluted blood at low flow rates and can be used for ~8 hours without clogging.

KEYWORDS: Platelet enrichment, Radial pillar device, Clogging-free, Microfluidic sorting

INTRODUCTION

Pillar-based sorting devices are useful for separating cells with different sizes. However, larger cells often clog the device, limiting the filter operation. The efficiency of pillar-based devices is further affected by the deformability of cells [1]. Platelets (1-3 µm) can be separated from deformable RBCs (7-8 µm) using pillar gaps of ~1µm. Such small features are often difficult to accurately fabricate. We report the use of a RAdial PIllar Device (RAPID) to separate platelets from blood with no clogging of the device. The specific arrangement of pillars sets up cross flows inside the device that prevents stacking of RBCs, thereby avoiding clogging. Our device can enrich platelets by 50-fold with a purity of ~70%.

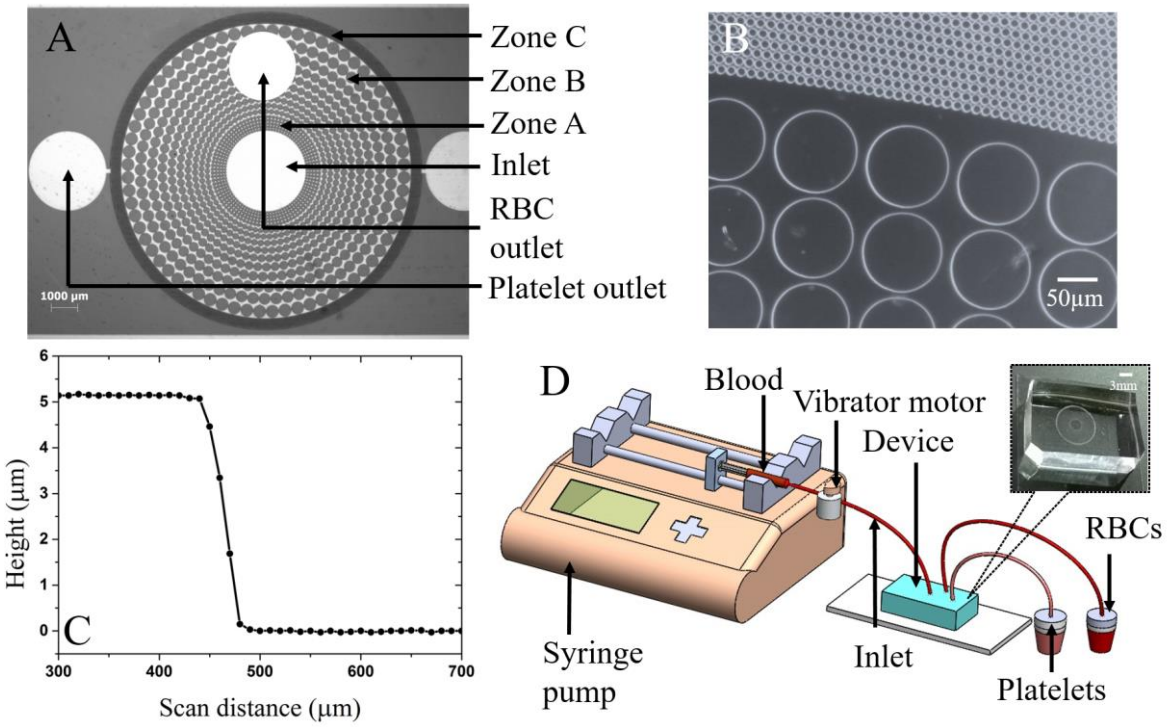

Figure 1: (A) Photo of RAPID showing concentric arrangement of pillars in different zones. Zone A blocks WBCs. Pillars in zone B have an angular displacement to create tangential cross flows. Zone C captures RBCs and allows the platelets to pass through. (B) Image of a part of the device showing the interface between zones B and C. (C) Profilometry data shows the device height to be 5µm. (D) Schematic diagram of the experimental setup.

EXPERIMENTAL

Blood was collected from a healthy individual and transferred to an EDTA-coated vacutainer tube. Sorting experiments were performed with blood diluted 20 times using 0.9% normal saline. The design of the micro-pillars in our device (Fig.1A) mimics a sunflower head. There is a central inlet to

add blood and two outlets at the periphery to collect platelets. Another outlet in zone B collects the RBCs (RBC outlet). Pillars in zone A blocks WBCs. The successive rows of pillars in zone B are displaced by an angle to set up strong tangential flows towards the RBC outlet. The pillars in zone C have a gap of 2µm to allow the platelets to pass through, while blocking the RBCs at low flow rates. The height of the device is 5µm (Fig.1C) to avoid flipping of RBCs. Fig.1D shows the schematic diagram of the experimental set up. A vibrator motor is attached to the tubing to prevent the cells from settling over the duration of the experiment. Flow rates from 600nl/min to 10µl/min were explored to study the effect of flow rate on cell separation parameters. First, 50µl of sample was allowed to collect in the RBC outlet and the outlet was then clamped, thereby increasing the sample flow to the platelet outlets. Once 50 µl of sample was collected from the platelet outlets, all samples were counted using a calibrated hematology analyzer.

RESULTS AND DISCUSSION

The tangential flow in zone B carries most of the RBCs and some of the platelets to the RBC outlet. Therefore, the platelets reaching the radial outlet are relatively pure. Fig.2 shows the enrichment factor and the purity of the platelets as a function of flow rate. For platelets, purity (65%) and enrichment (~ 50 fold) are the highest at the lowest flow rate (600 nl/min), since most RBCs travel towards the RBC outlet along the cross flow. Increasing the flow rate forces the deformable RBCs to squeeze through the small pillar gaps in zone C towards the platelet outlets, reducing separation purity.

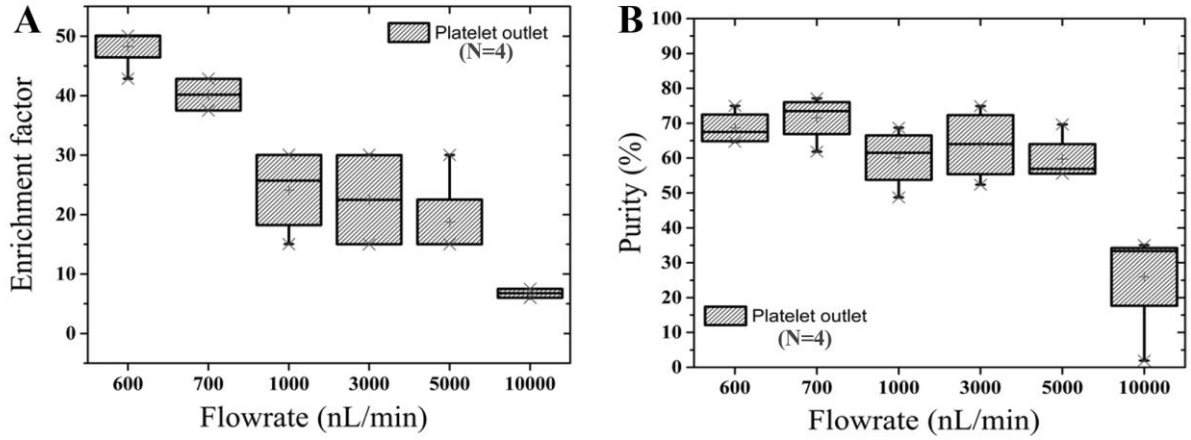

Figure 2: (A) Platelet enrichment decreases with increase in flow rate. (B) The separation purity of platelets decreases from 75% to 20% with increase in flow rate. The reason for both these phenomena is that more and more deformable RBCs manage to squeeze through zone C towards the platelet outlets with increase in pressure.

CONCLUSION

The device was tested for continuous operation (up to ~ 8 hours) without any clogging issues. The platelets were enriched up to 50 times at low flow rates. As the flow rate increases, RBCs squeeze through smaller pillar gaps meant for passing platelets. Our device performs the best under low flow rates, where platelet activation is avoided.

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REFERENCES

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