ISOLATION OF WHITE BLOOD CELLS USING PAPER-TRIGGERED DISSOLVABLE-FILM VALVES ON A CENTRIFUGAL PLATFORM

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ABSTRACT

The inherent centrifugation capability of the so-called 'Lab-on-a-Disc' (LoaD) platforms is widely used for blood processing during sample preparation. Here we introduce a valving technique which enables rotational control of paper wetting to actuate dissolvable film (DF) valves. This mechanism is applied to the separation of whole blood into its chief constituents; plasma, leukocytes and erythrocytes.

KEYWORDS: Lab-on-a-Disc; Valving; Flow Control, Blood Separation; Centrifugal Microfluidics; Paper Microfluidics

INTRODUCTION

The centrifugal microfluidic concept has frequently been employed for blood processing [1-2]. Specifically common bench-top Density Gradient Centrifugation (DGC) has been adopted to these so-called "Lab-on-a-Disc" (LoaD) systems to isolate Peripheral Blood Mononuclear Cells (PBMCs) [2-4]. In DGC whole blood is initially layered on the density gradient medium (DGM) Ficoll. Under the impact of a centrifugal field, the denser red blood cells (RBCs) sediment through the DGM while the PBMC layer and plasma remain stratified above.

For integrated plasma and PBMC layer extraction, we introduced a centrifugo-pneumatic siphoning scheme [4] which allows to run DGC at high spin rates (60 Hz ≈ 400 g) while maintaining a residual spin rate of 2.5 Hz during extraction. These high spin rates reinforce the centrifugally induced layering and thus improve the quality of the extract. Unlike other schemes, no hydrophilization of the siphon channel is required, therefore reducing complexity of manufacture and enhancing shelf life of the disc. However, efficient extraction of the PBMC layer was compromised during the slow-spin phase (siphon priming) as the centrifugal field stabilizing the PBMC layer is attenuated; this tends to distort the PBMC band as the centrifugal fields points out of plane (Fig. 1).

We therefore introduce a new centrifugal method for dividing blood into its two main cellular constituents and plasma where the three layers remain well defined by maintaining a continuously high spin rate throughout the process. We leverage the properties of paper, where wicking is moderated by the centrifugal force [5] to trigger our recently introduced [6,7], event-triggered dissolvable-film (DF) valves.

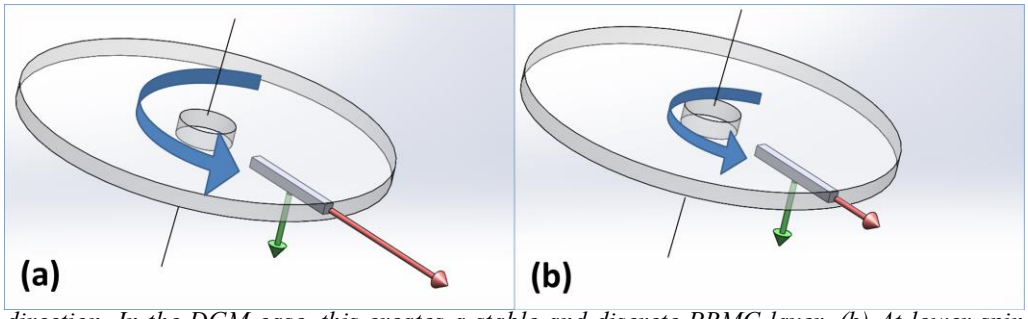

Figure 1 – Gravity and the centrifugal field acting on liquids stored on-disc. (a) At high spin rates the centrifugal force (red) prevails and the total force vector acts in the radial direction. In the DGM case, this creates a stable and discrete PBMC layer. (b) At lower spin rates the centrifugal force reduces and gravity (green) becomes dominant. Thus the total force vector reorients in towards the axial direction. In the 'bench-top' case, DGM is performed using a flying-bucket centrifuge. For on-disc DGM, this effect requires valves which actuate at high spin rates, thus, for example, ruling out the use of siphon valves.

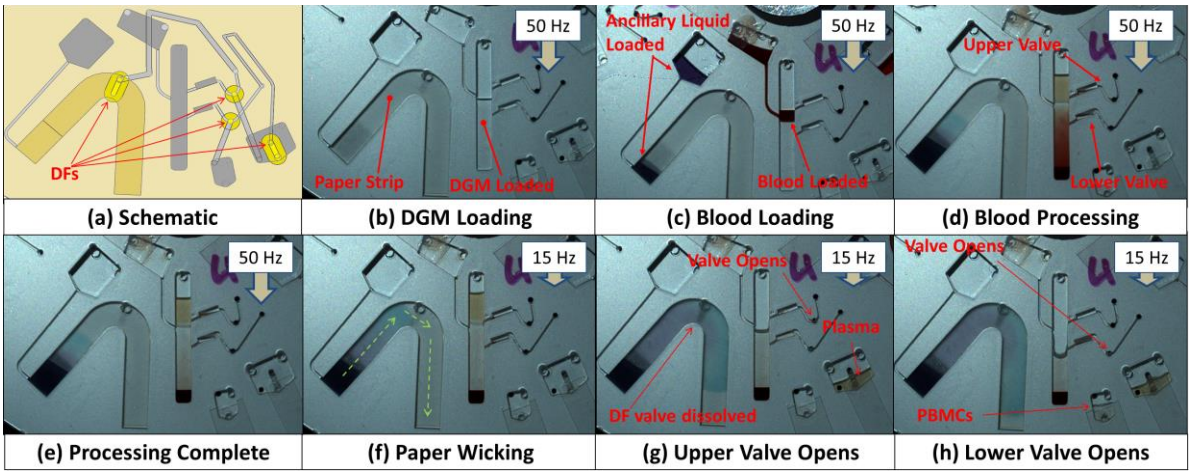

Figure 2 – Schematic and images of the low-pass variant valve. The valve uses an ancillary liquid, wicking through a paper strip, to open the valves. As the wicking can be controlled by the spin rate this valve is rotationally controlled. Here, the triggering DF is located more central along the paper strip. At high spin rates (~50 Hz) the liquid cannot progress inwards. (b-d) Blood is loaded and processed (e) Upon completion of blood processing the spin rate is reduced to ~15 Hz which is still high enough to ensure the PBMC layer remains intact (f). At the lower spin rate the ancillary liquid wicks inwards and actuates the first event-triggered valve. (g) The upper valve opens removing plasma. (h) The lower event-triggered valve CF is located in the plasma reservoir; this, in turn, opens the lower valve and removes the PBMCs to a second collection chamber.

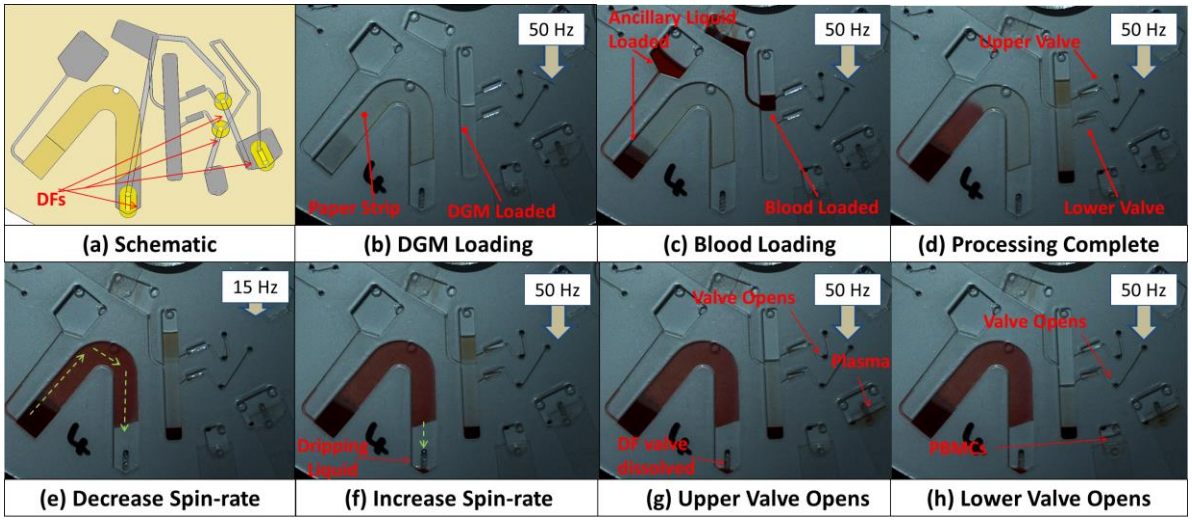

Figure 3 – Schematic and images of the high-pass variant valve. The valve uses an ancillary liquid wicking through a paper strip to open the valves. As the wicking can be controlled by the spin rate this valve is rotationally controlled. Here, the triggering DF is located radially outward below the paper strip. At high spin rates (~50 Hz), the liquid cannot progress inwards. (b-d) Blood is loaded and processed. (e) Upon completion of blood processing the spin rate is reduced to ~15 Hz which is still high enough to ensure the PBMC layer remains intact. In parallel, the ancillary liquid wicks inwards and wets the entire paper strip at this lower spin rate. The paper has sufficiently high capillarity that the liquid cannot drip from it at this low spin rate.?] (f) The spin rate is increased and liquid drips from the paper strip and wets the CF of the first event-triggered valve. (g) The upper valve opens to remove the plasma. (h) The lower, event-triggered valve CF is located in the plasma reservoir; this, in turn, actuates the lower valve and forwards the PBMCs to a second collection chamber.

MATERIALS AND METHODS

The discs used were manufactured using previously established multilamination [6]. The microfluidic structures in Figures 2 and 3 are composed of a sedimentation chamber with upper and lower valves for extracting the plasma and PBMC layers, respectively. A second chamber contains a siphon-shaped paper

strip, first oriented radially inwards and then outwards. Loaded simultaneously, dyed water wets the radially outward section of the paper as blood is layered onto the DGM. At high spin rates, the blood is processed but the liquid front in the paper cannot advance radially inwards at high spin rates due to the counteracting centrifugal field.

In a first configuration (Fig. 2) the valve is triggered by reducing the spin rate. The liquid front wicks inwards and can wet a DF, thus triggering the upper valve. The extraction of plasma through this valve then opens the lower valve, hence resolving the PBMCs to a collection chamber.

In an alternative configuration, the triggering DF is located radially outward of the paper strip. In this case, the valve is initiated by lowering the spin rate, causing the paper to absorb the dyed water; upon a subsequent increase of the spin rate, a small liquid volume is expelled from the paper to wet the triggering LF. Thus, the process is controlled by a transient reduction of followed by an increase in the spin rate.

RESULTS AND DISCUSSION

The interplay of the centrifugal force and capillary wicking through paper to actuate DF valves offers a number of advantages. As primary benefit, the actuation of the valves can, in both schemes, be implemented at greater spin rates than established low-pass valving techniques such as capillary or centrifugo-pneumatic primed siphons. This ensures that the PBMC layer remains is centrifugally well stabilized and correctly oriented during the extraction. These alternative approaches also permit the flexibility to extract the PBMCs at moderate (Fig. 2) or elevated spin rates (Fig. 3).

CONCLUSION

We present a technique based on rotationally controlled paper imbibition for the separation of whole blood. Unlike siphon or CPSV based extraction, this rotationally actuated valving scheme can operate at elevated spin rates in order to retain layer integrity. As a result, highly concentrated PBMCs are purified, which is pivotal for most cell-based diagnostics. The next step is to study the relative efficiency of extraction (based on moderate or high spin rates) and to integrate the PBMC extraction into sample-to-answer devices for applications such as rare cell detection and analysis.

ACKNOWLEDGEMENTS

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