MICROFLUIDIC APP FOR BUFFY COAT EXTRACTION FROM LARGE PERIPHERAL BLOOD SAMPLES FOR LOW-ABUNDANCE LIVING-CELL ANALYSIS

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ABSTRACT

A fully-automated centrifugal App for the isolation of two fractions, plasma and living cells, from a 2 mL peripheral blood sample is presented. In contrast to existing technologies, our App enables fully-automated processing on a standard laboratory centrifuge. It covers plasma separation and extraction, 1.7 mL density gradient medium (DGM) underlay, density gradient centrifugation (DGC) and extraction of the buffy coat fraction. The complete extraction takes 34 min and was successfully demonstrated for the clinically relevant hematocrit (HTC) range of 25 – 55 %.

KEYWORDS: Centrifugal, Buffy coat, Cell isolation, Separation, Blood, Low-abundance cells

INTRODUCTION

Aiming at the development of a pre-analytical application for the isolation of rare cells such as circulating tumor cells (CTCs), the low-abundance of <1000 target cells/mL demands handling of samples in the mL range [1]. Centrifugal microfluidic operations for isolation [2] and combined analysis of rare cells [4] have been presented. However, those solutions are either designed for much smaller sample volumes or require additional instrumentation. Our App enables the handling of mL volumes covering the clinically relevant HTC range of 25 – 55 % and is designed for being processed on a standard centrifuge (Z326K, Hermle, Germany) using passive liquid routing.

MATERIALS AND METHODS

Based on the results generated in network simulation (MatLab Simscape), the CAD-designed layout was micro-milled in a PMMA substrate (t = 10 mm; $Ø$ = 135 mm). The structured substrate was subsequently sealed with a pressure-sensitive tape (HJ-Bioanalytik, #900320), and mounted onto a rotor designed for the Hermle Z326K centrifuge (fig. 2-left). For monitoring of the cell viability, KG-1 cells (DSMZ#: ACC 14) were fluorescently labeled using the Cell-tracker™ CMFDA dye (Life technologies, USA) according to the manufacturer's instructions and spiked into a whole blood sample in a final concentration of 7.75 x 10^4 cells/mL and carried through the whole process.

EXPERIMENTAL

After mounting the platform onto the rotor, the inlet chambers were loaded with 2.0 mL of peripheral blood and 1.7 mL DGM (Ficoll-Paque PLUS, GE), respectively (fig. 1-0 & fig. 2-left). Upon starting the spinning protocol, the peripheral blood was transferred into the separation chamber, where the plasma was separated at RCF = 400 g (approx. 40 Hz at r = 65 mm) for 15 min. Simultaneously, the timer structure [5] was loaded with the DGM (fig. 1-1). After separation, 600 µL of plasma were isolated into a plasma collection reservoir at 20 Hz (fig. 1-2) before the timer was released at 5 Hz followed by a transfer of 1.5 mL DGM into the blood inlet using centrifugo-dynamic inward pumping (CDIP) [6] (fig. 1-3). Upon acceleration to 400 g, the DGM was transferred into the separation structure underlaying the red blood cells (RBCs) and lifting the buffy coat fraction above the radial position of the cell extraction siphon. After 15 min DGC at 400 g (fig. 1-4 & fig. 2-right), the plasma/buffy coat/DGM fraction was extracted at 5 Hz and collected in the extraction chamber (fig. 1-5 & fig. 3-left image). Microscope images using simultaneous fluorescent and bright field imaging of the extracted fraction showed fluorescing (and thus viable) KG-1 cells and characteristic halo artifacts of (viable) white blood cells (WBCs) in the background, as depicted in fig. 3-right.

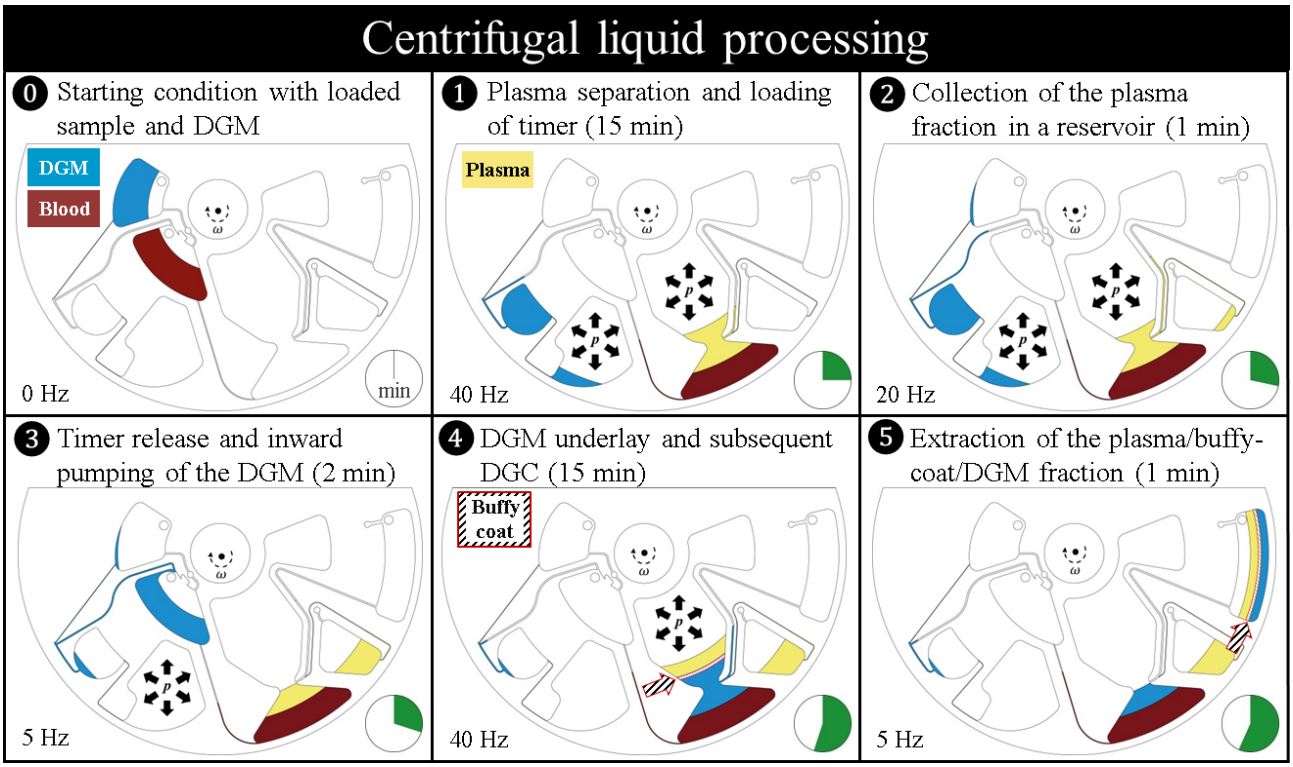

Figure 1: *Centrifugal processing of the blood sample and DGM. After plasma separation (1), the RBCs were underlaid with the DGM followed by the DGC step (4) and the extraction of the plasma/buffy coat/DGM fraction, which could be collected from the collection chamber for subsequent analysis steps. Generated overpressures in chambers are marked with arrows.*

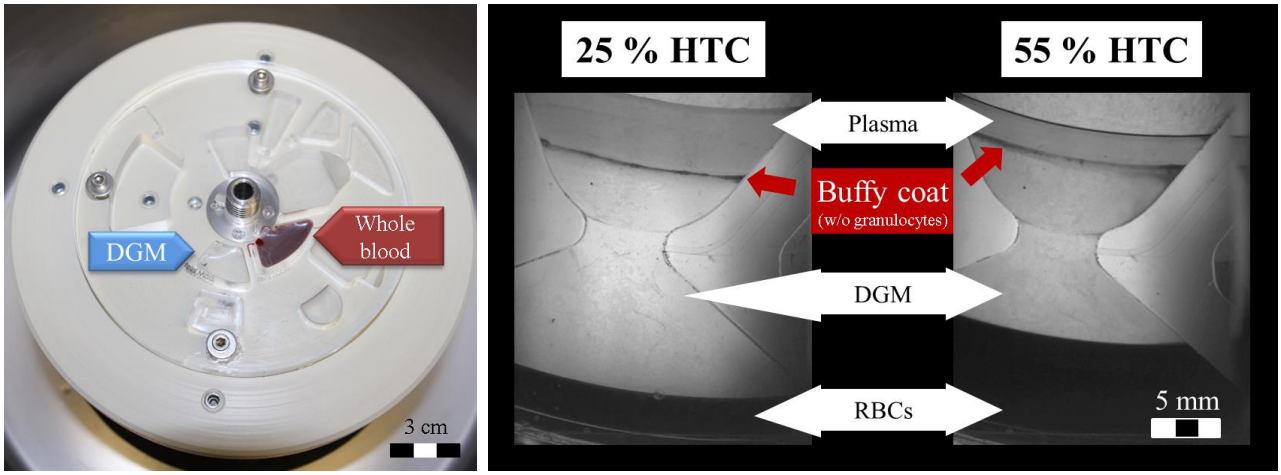

Figure 2, left: *Image taken after mounting the Disk onto the rotor in the centrifuge and loading with a peripheral whole blood sample and DGM (step 0 in fig. 1).* ***Right:*** *Images taken after 15 min of DGC on a centrifugal test-stand (step 4 in fig. 1) from blood samples with 25 and 55 % HTC, respectively, with the buffy coat layer marked with red arrows located well above the radial position of cell extraction siphon.*

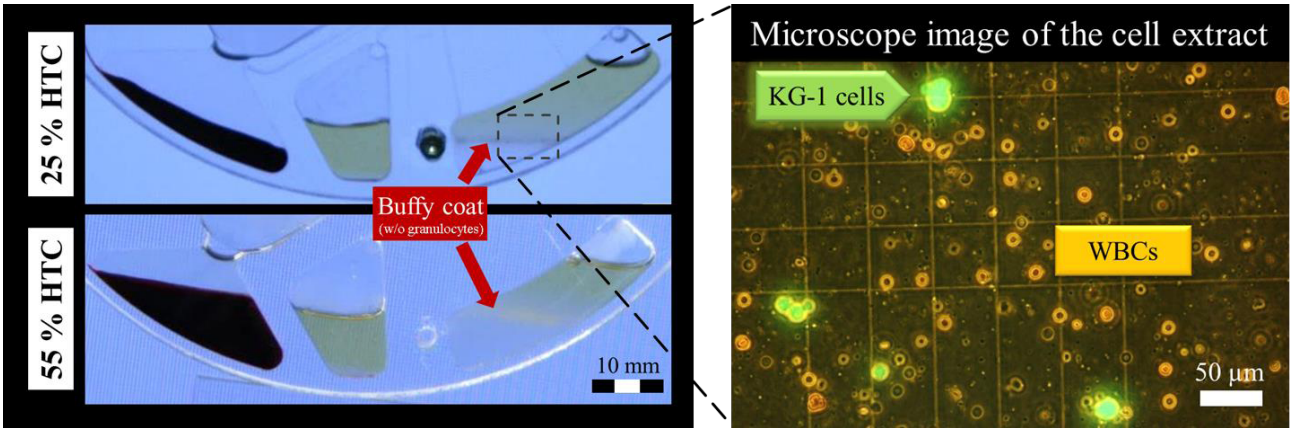

Figure 3, left: *Images taken after the DGC (step 5 in fig. 1) from blood samples with 25 and 55 % HTC, respectively, with the buffy coat layer marked with red arrows.* ***Right:*** *Microscope image of the extracted plasma/buffy coat/DGM fraction using both fluorescent and bright field imaging, whereas the fluorescent light dots of the CMFDA dye indicate viable KG-1 cells. Other human WBCs also show viability-characteristic halo formation in the back.*

CONCLUSION AND OUTLOOK

A centrifugal App for fully-automated extraction of plasma and concentration of low-abundance cells has successfully been demonstrated. The time-to-result of 34 min is in the range of the manual process (DGM with Ficoll according to manufacturer's instruction: 30 min) yet not requiring any manual steps. Fluorescently marked KG-1 cells have proven to survive the entire isolation process, which is an indicator for later rare cell isolation. As a next step, we aim at reducing the overall processing time below 15 min. Subsequently, a magnetic-bead assay for the specific labelling and isolation of living CTCs will be implemented, which then are available for further analysis. As an add-on, the collected plasma fraction allows for additional parameter analysis.

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REFERENCES

[1] U. Dharmasiri *et al.* Microsystems for the Capture of Low-Abundance Cells. *Annual Review of Analytical Chemistry* **3,** 409–431 (2010).

[2] D. J. Kinahan *et al.* Spira mirabilis enhanced whole blood processing in a lab-on-a-disk. *Sensors and Actuators A: Physical* **215,** 71–76 (2014).

[3] D. Kirby *et al.* Rapid and cost-efficient enumeration of rare cancer cells from whole blood by low-loss centrifugo-magnetophoretic purification under stopped-flow conditions. *Cytometry A* **87,** 74–80 (2015).

[4] J.-M. Park *et al.* Fully automated circulating tumor cell isolation platform with large-volume capacity based on lab-on-a-disc. *Analytical Chemistry* **86,** 3735–42 (2014).

[5] Schwemmer, F. *et al.* A microfluidic timer for timed valving and pumping in centrifugal microfluidics. *Lab on a Chip* **15,** 1545–1553 (2015).

[6] Zehnle, S. *et al.* Centrifugo-dynamic inward pumping of liquids on a centrifugal microfluidic platform. *Lab on a chip* **12,** 5142–5 (2012).

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