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AQUEOUS TWO-PHASE SYSTEM-ASSISTED BLOOD FRACTIONATION AND CELL ISOLATION IN CENTRIFUGAL MICROFLUIDICS

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ABSTRACT

We present an automated centrifugal microfluidic platform (CMP) for whole human blood fractionation and blood cell isolation applications using an aqueous two-phase system (ATPS). The advanced ATPS layers are obtained from a polyethylene glycol (PEG) - dextran (DEX) mixture and enable to fractionate the whole blood as a density gradient media. We show that the CMP comprised with the applied centrifugal force and pneumatic pressures is able to extract the fractionated blood cells to an *in-situ* separation fashion.

KEYWORDS: Centrifugal Microfluidics, Aqueous Two-Phase System, Blood Fractionation, Cell Isolation

INTRODUCTION

Fractionation of whole blood components is an important process in many clinical applications.[1] The existing blood fractionating methods are dependent on laboratory protocols that use serological pipettes requiring gently layering the blood sample on the density media followed by centrifugation and extraction of the components of interest. However, the sample handling process is performed manually and pipetting-based steps are to be avoided to obtain reliable and reproducible results. Here, we demonstrate an automating centrifugal microfluidic approach of the entire blood fractionation and extraction steps using ATPS that can be a density-matched solution with the targeted blood cell types.

EXPERIMENTAL

We used a custom-made mixture of ATPS to create advanced separation layers. A mixture of stock solutions, PEG (5 w/v %, Mw 35k) and DEX (7 w/v %, Mw 500k), are used for our experiments.[2] We also added sodium diatrizoate to match the density of blood cells, which leads the densities of PEG and DEX to 1.071 and 1.086 g/cm^3, respectively. This density gradient media is able to extend the fractionation band of blood samples and can be dynamically tuned for a variety of blood components beyond the usual white blood cells (WBCs) and red blood cells (RBCs).

The microfluidic device was designed and fabricated in-house exclusively by CNC machining, injection molding and 3D printing. The main chip is composed of several fluidic reservoirs (ATPS, high density gradient media, waste, separation and collection chambers) as well as channels connecting these reservoirs mutually with the world-to-chip interface (Fig. 1).

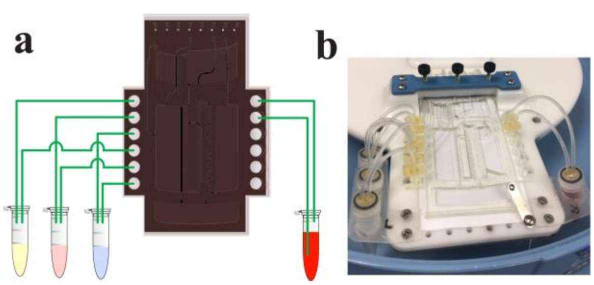

Figure 1: (a) Schematic design and configuration of the blood fractionating microfluidic device. The design consist of microchannels, reservoirs and holes. The device is produced using a micromilling machine. (b) A fabricated microfluidic chip is mounted on the CMP. Eppendorf tubes are interfaced with flexible tubing and positioned next to the chip for world-to-chip connection.

RESULTS AND DISCUSSION

We performed whole blood fractionation and isolation experiments on the CMP using ATPS as a density gradient media. The entire process is shown in Fig. 2. The ATPS mixture is first transported to the fractionation chamber and phase-separated with the applied centrifugal force. Then, we layer the whole blood over the ATPS solution by activating the port connected to the Eppendorf tube (Fig. 2a). After centrifugation, we clearly observe five distinct layers in the fractionation chamber i.e., plasma, buffy coat, PEG, DEX and RBCs (Fig. 2b). To isolate these different layers, we move the top layer of plasma situated above the extraction channel inlet into the intermediate collection chamber by applying a positive pressure on the fractionation chamber. For other cell layers, we propose high density gradient media (HDGM, 1.324 g/cm^3) and a special microfluidic structure to push the ATPS and blood in the fractionation chamber (Fig. 2d). Subsequently, peripheral blood mononuclear cells (PBMCs) and neutrophils layers are repositioned and transferred to the collections chambers as shown in Fig. 2e and Fig. 2f, respectively.

The isolated cells are stained with fluorophore-conjugated antibodies and counted under the fluorescence microscope. For this proof-of-concept study, we collect three different layers of plasma, PEG with PBMCs and DEX with neutrophils. Samples lies in the microfluidic collection chamber are transported to the each individual Eppendorf tube by applying negative pneumatic pressures (Fig. 3a). Fig. 3b shows the results of stained images of platelets, leukocytes and neutrophils. For the platelet-rich layer, we do not find any significant number of WBCs and RBCs. Fig. 3c shows the number of stained PBMCs and neutrophils against cell concentration.

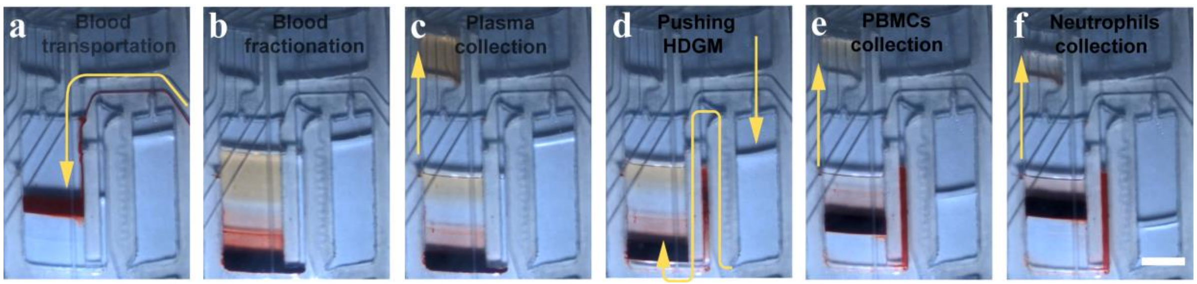

Figure 2: Stroboscopic images of different steps of whole blood fractionation and cell extraction. (a) Blood transportation to the microfluidic device after ATPS separation. (b) Fractionated four different blood layers; plasma, buffy coat (PEG), neutrophils (DEX) and RBCs. (c) The plasma layer is moved to collection chamber. (d) Moving the layers up. HDGM is pushing the whole blood layers. (e) The PBMCs are transported to the collection chamber. (f) The neutrophil cells are transported to the collection chamber. Scale bar is 10 mm.

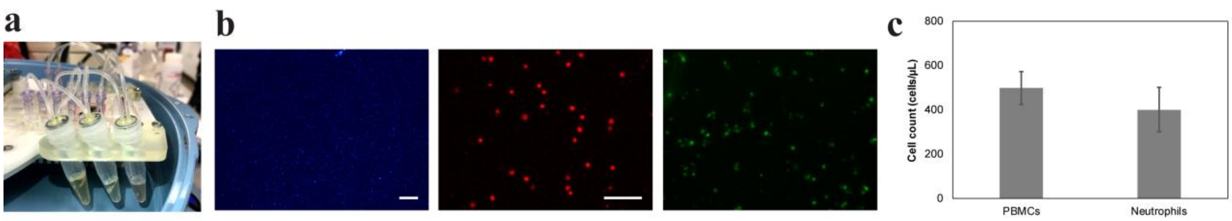

Figure 3: (a) Extracted samples from the Fig. 2 CMP operated protocol. (b) Stained images of platelets (hoechst staining, left), leukocytes (CD45, middle) and neutrophils (CD15, right). Scale bars are 100 µm. (c) A graph of counted cell numbers of PBMCs and neutrophils.

CONCLUSION

We have demonstrated that the advanced layers of ATPS enables to fractionate whole blood and isolate blood cells on the CMP. This automating ATPS-CMP system can find in applications for cell isolation procedures and bioanalytical assays.

ACKNOWLEDGEMENTS

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