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ENRICHMENT OF PERIPHERAL BLOOD MONONUCLEAR CELLS FROM LARGE VOLUMES OF BLOOD USING CENTRIFUGAL MICROFLUIDICS

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ABSTRACT

A fully automated microfluidic cartridge for efficient and high-throughput isolation of PBMCs with high viability and purity is presented. The proposed approach uses a centrifugal microfluidic platform with pneumatic actuation and world-to-chip interfacing for accurate control of blood fractionation processes, allowing precise metering and extraction of PBMCs from large volumes of blood samples in a sequential manner. The disposable passive cartridges were fabricated in standard thermoplastic polymers, which allows future use of scalable manufacturing methods suitable for industrial translation.

KEYWORDS: blood fractionation, centrifugal microfluidics, active pneumatic pumping, world-to-chip interface.

INTRODUCTION

Enrichment of peripheral blood mononuclear cells (PBMCs) from whole blood is at the heart of many downstream applications including cell therapies. When *in-vitro* culture follows PBMCs isolation, a reliable label-free collection approach that can viably and appropriately isolate PBMCs from relatively large volumes of blood is required. Although microfluidic technology offers advantages over macroscale methods, such as decreased reagent consumption, gentler cell handling and compatibility with automation among others, it is rather limited to relatively small sample volumes (usually few hundreds of microliters) [1]. In this paper, we propose a centrifugal microfluidic approach to efficient isolation of PBMCs from large volumes of blood (>5 mL) with high viability and purity which takes advantage of active pneumatic pumping principles [2] and multistep blood fractionation.

EXPERIMENTAL

The microfluidic cartridge was fabricated in a 4-mm thick Zeonor substrate using CNC machining with features carved on both sides (Fig. 1a-b) and sealed using adhesive and flat Zeonor substrates. The cartridge consists of a blood separation chamber (SC), a cell collection chamber (CC), a heavy oil chamber (HO) and an intermediate heavy oil chamber (IHO) (Fig. 1c). Blood, Ficoll, waste, plasma and cell collection vials are external and fluidically connected to the chip through a world-to-chip interface ports (A1-6 and B1-6) with several fluidic conduits controlled by applying pulses of pressure to the ports (1-12) of the microfluidic device while the platform is rotating. Once placed on the platform, the cartridge is rotated at 500 rpm and pneumatic actuation activated at selected ports to transfer liquids to and from the cartridge [2]. The blood in the external vial is processed sequentially in multiple steps, each of them consisting of (Fig. 1d): (I) transferring 400 μL of Ficoll from the external tube to the SC; (II) layering 600 μL of blood on top of the Ficoll; (III) blood fractionation for 15 minutes; (IV) plasma isolation and transfer to the CC; (V) plasma transfer from the CC to the external tube; (VI) heavy oil transfer from HO through the IHO to the bottom of the SC to precisely position the buffy coat at the entrance of the extraction conduit; (VII) PBMCs transfer to the CC; (VIII) PMBCs transfer from the CC to the external tube; (IX) homogenization of remaining liquid in the SC by bubble mixing; (X) emptying of SC chamber (to external waste tube) enabling next processing step. The process is then repeated to allow processing of 5 mL of blood.

RESULTS AND DISCUSSION

Yield, viability and purity of isolated PBMCs from commercially available blood using automated microfluidic platform was compared to manual density gradient centrifugation. For the same blood volume, a similar number of recovered PBMCs was obtained using both methods (Fig. 1e). A viability experiment was also performed using a live/dead fluorescence assay to reveal that viability of isolated PBMCs is better preserved using automated isolation method (85%) compared to manual approach (56%). Finally, purity of the PBMCs is evaluated using Vybrant DyeCycle Green DNA stain allowing enumeration of the nucleated cells. The microfluidic cartridge provided much higher purity of PBMCs (92%) compared to manual method (36%) (Fig. 1e-g).

CONCLUSION

An automated microfluidic platform and microfluidic cartridge based on active pneumatic pumping is presented and validated for PBMC isolation from whole blood and large volumes of sample (5 mL). When compared to standard manual protocols, higher cell purity and viability is obtained.

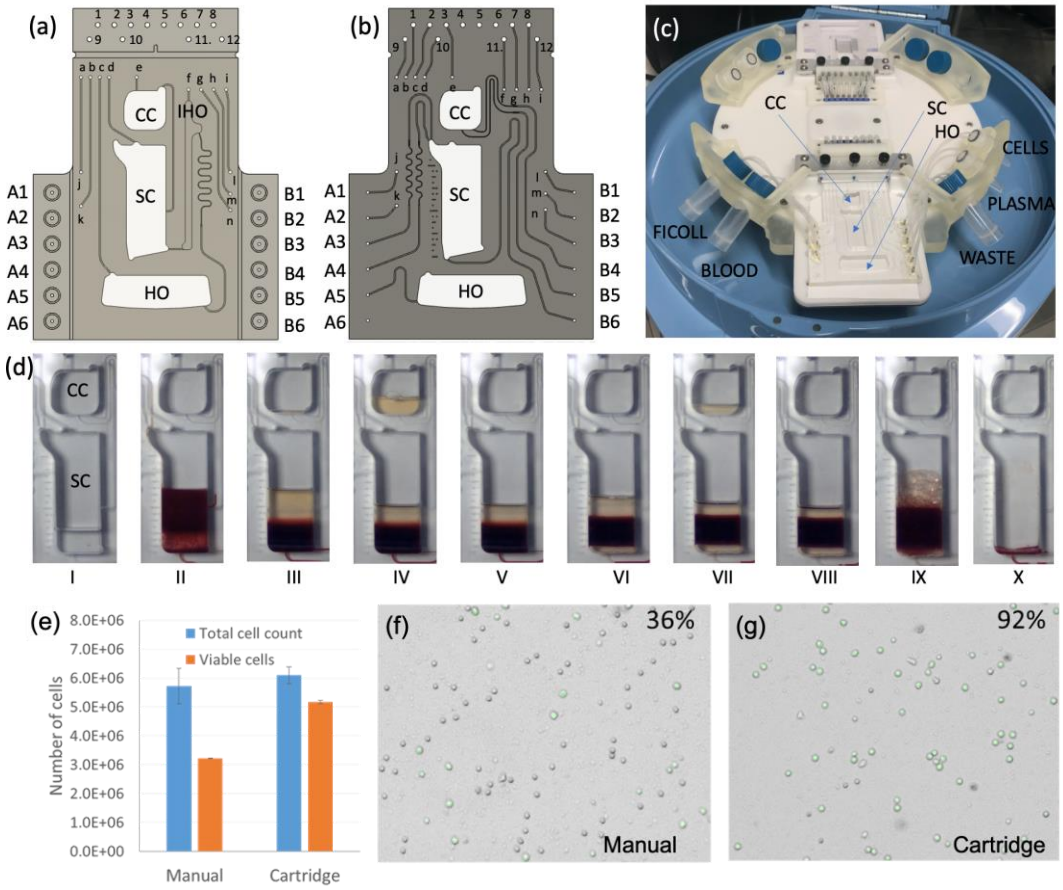

Figure 1: Top (a) and bottom (b) microfluidic levels with pneumatic actuation ports (1-12), world-to-chip interface (A1-6, B1-6) and thru holes (a-n) for fluidic communication between the two levels. The separation chamber (SC), the heavy oil chamber (HO), the intermediate heavy oil chamber (IHO) and the collection chamber (CC) are also indicated; (c) Photograph of the fabricated microfluidic chip installed on the rotor of the centrifugal platform and connected to the external reservoirs; (d) Sequence of stroboscopic images featuring the separation (SC) and collection chambers (CC) at different stages of the blood fractionation process. (e)Yield and viability of isolated PBMCs comparing automated cartridge and manual density gradient centrifugation. Sample images showing purity of the isolated PBMCs comparing the standard manual procedure (f) to the microfluidic approach (g) proposed here.

ACKNOWLEDGEMENTS

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