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PROGRAMMABLE DENSITY-BASED FRACTIONATION IN CENTRIFUGAL MICROFLUIDICS

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ABSTRACT

This paper presents a method to precisely control the process of fractionating a wide range of samples in centrifugal microfluidic devices with on-the-fly compensation for intrinsic sample-to-sample variability. We show precise control of the position of fractionated layers through the programmable pneumatic-based dispensing and removal of a high density liquid to/from the bottom of a separation chamber. The high density liquid generates a displacement of the fractionated layers in the radial direction, allowing precise alignment with an outlet channel and accurate extraction of specific components despite variability in the composition of the raw sample.

KEYWORDS: Centrifugal Microfluidics, Fractionation, Active Pneumatic Pumping, Blood

INTRODUCTION

One key advantage of centrifugal microfluidics resides in its capacity to perform on-chip sample preparation steps through density-based fractionation. Typically, a complex raw sample (such as blood, cell culture, soil sample, etc.) is introduced in a dedicated microfluidic chamber where the high centrifugal acceleration enables efficient separation of its various components based on their respective density. One or more outlet channels then allows recovery of specific fractions of the sample for further on-chip processing. Unfortunately, this classical approach suffers from a key practical limitation: as the position of the outlet channels is fixed, intrinsic sample-to-sample variability can lead to significant changes in the position of the target fractions, limiting the precision and repeatability of the extraction process. This is particularly problematic when the target fraction represents a small proportion of the original sample (e.g. buffy coat extraction from blood) or when multiple targets must be precisely separated despite small density differences (e.g. isolation of target cells from a large population). In this paper, we demonstrate a method to precisely tune on-the-fly the radial position of the various layers of a fractionated sample based on the dispensing of a high density liquid to the bottom of the fractionation chamber using a centrifugal microfluidic platform with active pneumatic control.

EXPERIMENTAL

All the experiments were performed using a centrifugal microfluidic technology that was recently developed allowing independent control of the pneumatic pressure applied on eight pressure ports located on the microfluidic devices during high-speed centrifugation (Fig. 1a). [1] Compared to previous work, a key difference in the design of the microfluidic devices (Fig. 1b) is the inclusion of a chamber allowing transfer of a high density liquid to the bottom of the separation chamber through a long serpentine channel by activation of pressure port #3. The design ensures that the flow of heavy liquid stops when pressure is released, allowing the end user to program on-the-fly the volume of heavy liquid dispensed. The microfluidic devices (Fig. 1c) were fabricated by CNC machining the channels and reservoirs in a 100 mm × 50 mm × 6 mm injected thermoplastic part (Zeonor 1060R, Zeon Chemicals). After machining, the

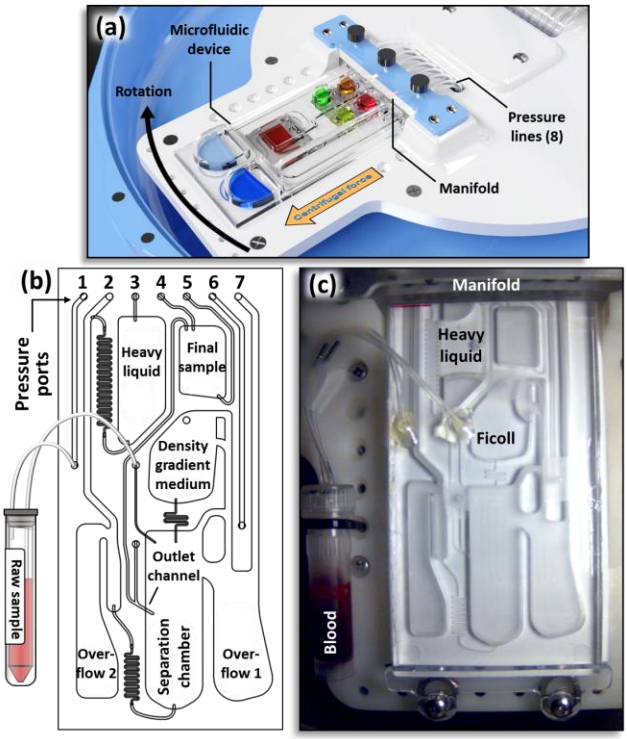

Figure 1: (a) 3D schematics showing the centrifugal microfluidic platform used for this study. (b) Schematics and (c) picture of the programmable fractionation microfluidic devices.

devices were closed with a thermoplastic elastomer bottom cover (Mediprene OF 400M, Hexpol TPE). Prior to the insertion on the platform, the devices were filled with 550 µL of a density gradient medium (Ficoll paque plus, Sigma-Aldrich) and 500 µL of a high density liquid (density of 2.85 g/cm^3, LST heavy liquid, Central Chemical Consulting). A 1 mL blood sample was inserted in a separate 2 mL vial connected to the chip using tubing to simplify sample loading and minimize risks associated with pipetting potentially pathogenic samples in a centrifugal microfluidic device. [2]

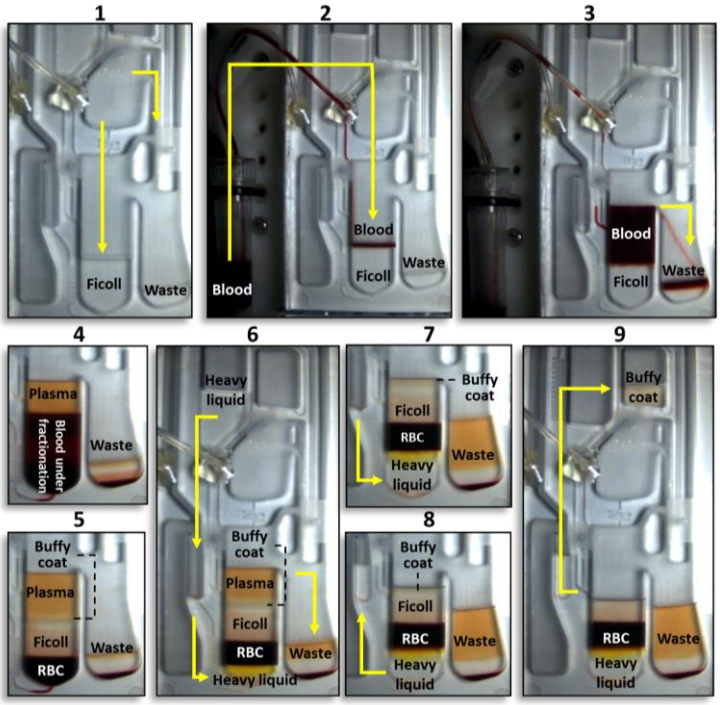

Figure 2: Sequence of images showing the various steps (from 1 to 9) of the assay with identification of the visible layers and fluidic transfer steps (yellow arrows).

RESULTS AND DISCUSSION

Figure 2 shows all the steps required to fractionate a blood sample, discard plasma, align the buffy coat with an outlet channel, and transfer it to a dedicated chamber. When the platform starts spinning (800 rpm), the centrifugal force enables the metering and transfer of Ficoll to the separation chamber (1). The pressure port #1 is then activated to load the blood on the chip, layer it on top of the density gradient medium (2), and meter a specific volume (3). After fractionation of the blood sample by centrifugation for 45 min at 800 rpm (4 and 5), heavy liquid is transferred to the bottom of the separation chamber by activating pressure port #3, resulting in a gradual displacement of the fractionated blood sample (6 and 7). No visible degradation of the fractionated layers was observed during this displacement. As the level of heavy liquid increases, the top layer of the fractionated blood sample (i.e. plasma) is gradually transferred to the overflow chamber #1. In this example, the heavy liquid transfer was stopped when the buffy coat layer nearly reached at the top of the separation chamber. It is important to note that, with the proposed concept, the displaced fractionated layers remain in stable equilibrium even when the pressure applied by the platform is released and heavy liquid transfer stops, allowing other fluidic operations to be completed if needed. The liquid level in the separation chamber was then lowered by activating simultaneously ports #4, 5 and 7, pushing the heavy liquid back toward overflow chamber #2. When the buffy coat layer nearly reached the level of the outlet channel (8), ports #4 and 5 were deactivated allowing transfer of the buffy coat at high concentration to the dedicated chamber (9).

CONCLUSION

The example provided herein demonstrates the capacity of this method to precisely control the radial displacement of fractionated layers relative to a collection point, opening the path to the on-chip integration of complex fractionation processes that remain efficient even for samples that are inherently highly variable.

ACKNOWLEDGEMENTS

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