HYBRID PAPER-POLYMER LAB-ON-A-DISC FOR BIOASSAY INTEGRATION

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ABSTRACT

In this work we present a novel, hybrid paper-polymer lab-on-a-disc system which combines both advantages of passive liquid movement in paper and active rotational flow control. The system enables both transport of plasma in the directions parallel or reverse to the centrifugal force and precise plasma dispensing by the spinning frequency. The system functionality has been demonstrated on the detection of triglycerides in plasma by implementing a commercial assay kit. To the best of our knowledge, this is the first time of a comprehensively integrated bioassay system in paper-polymer hybrid disc.

KEYWORDS

lab-on-a-disc, paper microfluidics, blood separation, colorimetric assay

INTRODUCTION

Nowadays, the high potential of paper-based, disposable point-of-care devices is undisputed due to its low cost [1-2], its amenability to dry reagent storage and surface functionalization [3], and for its ease of use even by unskilled users [4-5]. However, conventional paper microfluidics presents severe restraints due to merely imbibition based flow control. By synergizing the well-established lab-on-a-disc technology with paper microfluidics it is possible to overcome this significant bottleneck [6]. In the present work, contoured paper segments have been integrated into centrifugally actuated lab-on-a-disc platforms to take advantage of both autonomous capillary pumping and the particle filtering characteristics of paper [6-7]. This hybrid approach leverages a repertoire of liquid-handling operations (LUOs) such as flow control, filtering, metering or even liquid routing in a simple, cheap and fast fashion [7]. Particularly, we describe three fundamental microfluidic LUOs such as valving, blood separation and plasma metering. These processes would be very hard to engineer with conventional paper microfluidics. Moreover, we demonstrate the full integration of a colorimetric assay kit for the detection of triglycerides in blood.

MATERIALS AND METHODS

The hybrid lab-on-a-disc is made of Poly(methyl methacrylate) (PMMA), pressure sensitive adhesive (PSA) and chromatographic paper type 1 WHATMAN®. The PMMA parts were cut using a CO_2 laser, the PSA and paper siphons were defined using a knife cutter. All layers were consecutively stacked and sealed irreversibly. Figure1A shows a 3D-schematic of the hybrid disc assembly.

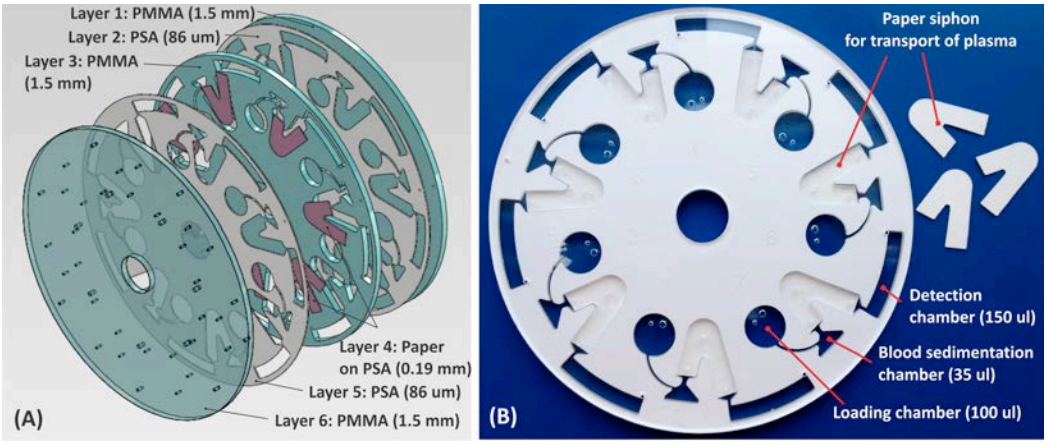

Figure 1. (A) 3D schematic of disc assembly. The device consists of 5 layers (3 layers of PMMA and 2 layers of double-sided adhesive, PSA). The Whatman® Chr 1 paper stripes with adhesive back are sandwiched between layer 3 and layer 1. (B) Picture of the final disc used for the final detection of triglycerides in blood.

Contoured paper segments are inserted in the siphon channels to provide centrifugo-capillary valving function. Figure 1B shows a photo of the disc used for the colorimetric detection of triglycerides in blood. For the experiments on detection of triglycerides in plasma, a CAYMAN® triglyceride colorimetric assay was sourced.

RESULTS AND DISCUSSION

Figure 2 depicts the two main drivers governing the novel valving mechanism: capillary action and centrifugal force. The main novelty is the use of paper as an active element for on-disc flow control and sample. At a threshold frequency of 3000 rpm (rotations per minute), the two forces are balanced to halt the flow. At elevated frequencies, liquid is centrifuged out of the paper (Fig. 2A). For lower frequencies capillary action prevails to propel liquid through the paper siphon towards the center of rotation (Fig. 2B). By repeatedly crossing the threshold frequency, we achieve liquid circulation though the siphoning channel. Capillary siphoning is a well-known valving technology, but the main innovation is the implementation of chromatographic paper as a capillary channel without requiring (typically unstable) hydrophilic surface modification to the polymeric channel.

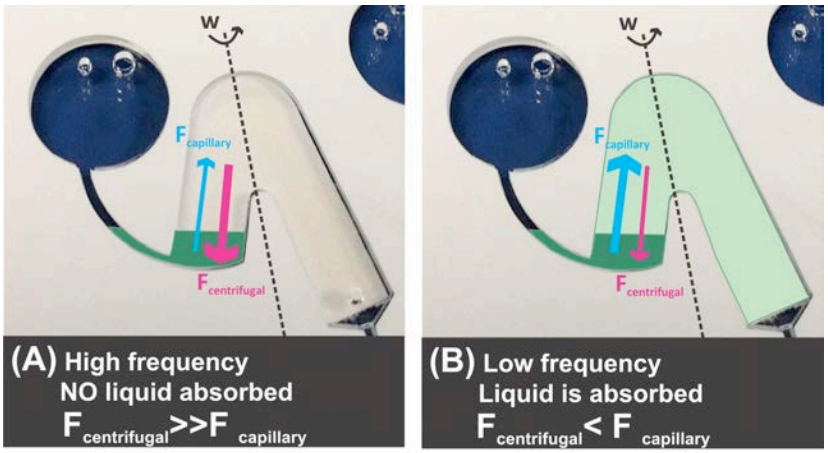

Figure 2. Schematic of the key counteracting driving mechanism, capillary action and centrifugation. (A) Above the threshold frequency, the centrifugal force suppresses inbound capillary flow. (B) Below the threshold frequency, capillarity dominates to drive the liquid through across the crest point of the paper siphon.

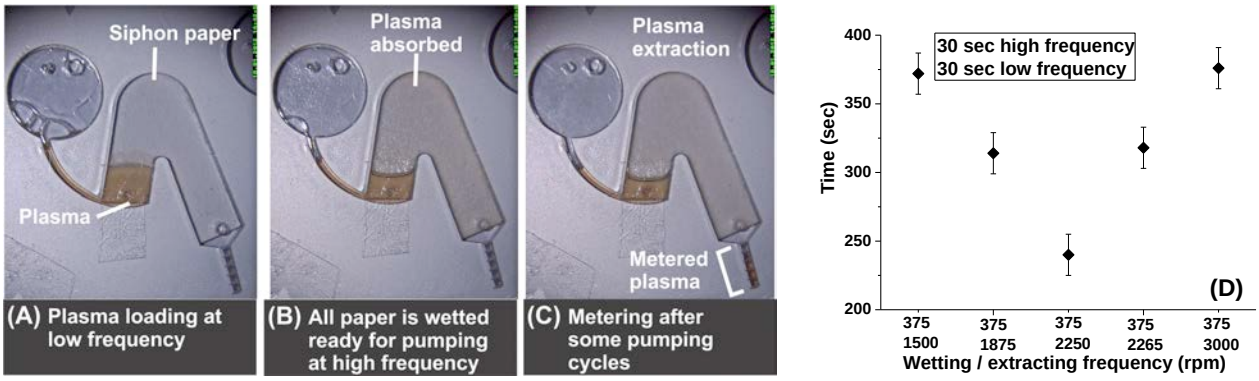

Figure 3.Plasma metering using centrifugo-capillary pumping cycles. (A) 70 μL of plasma is loaded at 375 rpm. (B) The plasma is continuously adsorbed but remains confined to the paper segment. (C) Plasma is extracted from the paper at high frequency (2250 rpm). After 4 cycles of 30 seconds at 375 rpm and 30 seconds at 2250 rpm 10 μL of plasma were extracted. (D) Optimization of the 10-μL plasma metering for at 375 rpm and a range of values for the upper frequency. The chosen cycle (375 rpm/2250 rpm) corresponds with the lowest plasma extraction time.

Figure 3 illustrates the fundamental microfluidic principle underlying accurate metering using centrifugo-capillary recirculation. After loading (Fig. 3A), the liquid is absorbed by periodic switching between low (capillary domain) and high (centrifugal domain) frequencies (Fig. 3B), and then expelled from the paper at high spin rates (Fig. 3C). In this example, 10 μL of human plasma, from an initial volume of 50 μL, was metered along four cycles of 30 seconds at 375 rpm and another 30 seconds at 2250 rpm with a precision of 1μL. The frequencies were optimized, as it is showed in Figure 3D, in order to minimize the number of cycles. Note that conventional lateral flow technologies do not permit the extraction of a metered volume from the paper membrane.

Using the optimized metering protocol, we automated paper-siphon based blood separation and colorimetric detection of triglycerides in whole blood using a commercial kit. Following the manufacturer's instructions, 10 µL of human plasma is incubated with an enzymatic mixture for 15 minutes. For that purpose, 80 µL of blood is loaded at 6000 rpm (Fig. 4A). At elevated frequencies (over the threshold frequency), red blood cells are separated in the designated sedimentation (Fig. 4B). Afterwards, the frequency of rotation is relaxed to 375 rpm and pure plasma is absorbed through the paper siphon (Fig. 4C). Using the already optimized pumping cycles 10 µL of pure plasma is metered and incubated with the enzymatic mixture, resulting in a color build-up from transparent to light purple (Fig.4D).

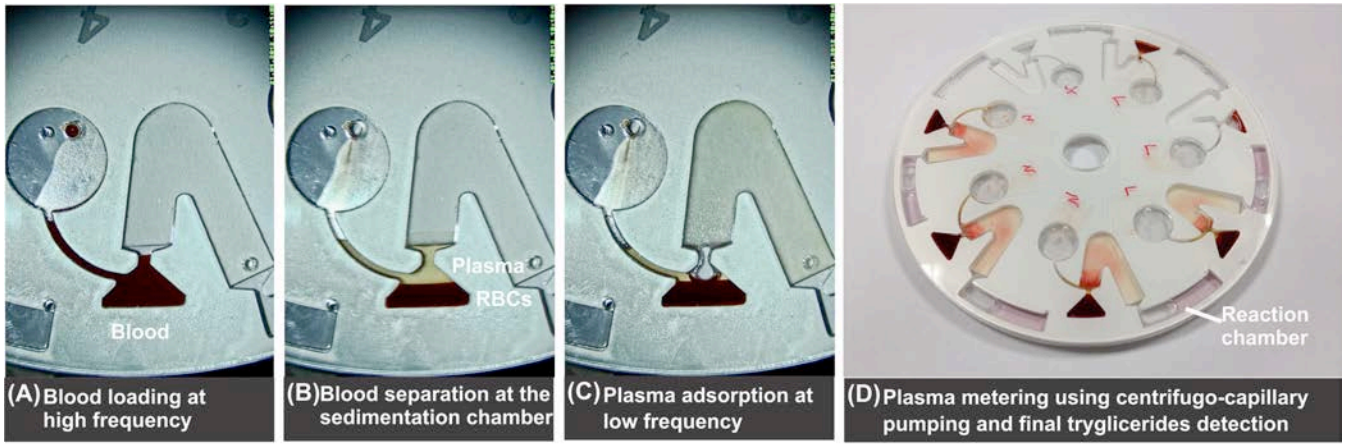

Figure 4. Colorimetric detection of triglycerides in blood. (A) 80 µL of human blood is loaded at 6000 rpm. (B) Whole blood separates in plasma and red blood cells. (C) When the frequency is relaxed to 375 rpm the plasma is absorbed through the paper siphon. The protocol for metering 10 µL uses cycling between 375 rpm and 2250 rpm. (D) After 15 minutes of incubation for the metered plasma and the enzymatic mixtures, a color change transparent to light purple is discernible.

CONCLUSIONS AND OUTLOOK

By integrating paper segments into a polymeric la-on-a-disc platform, we have integrated a colorimetric enzymatic assay relying on three fundamental LUOs: blood separation, plasma metering and valving. Encouraged by the high potential of such hybrid devices we will continue to extend the toolbox of centrifugal paper microfluidics towards more complex assay protocols, higher precision and sensitivity.

ACKNOWLEDGEMENT

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REFERENCES:

[1] G.M. Whitesides et al., *Nature*, **442**, pp. 368, (2006).
[2] D.Y. Stevens et al., Lab Chip, **8**, pp. 2038-2045, (2008).
[3] G.E. Fridley et al., Proc. µTAS 2011, Seattle, USA, pp. 981-983, (2011).
[4] E. Fu *et al*., Anal. Chem., **83**, pp. 7941, (2011).
[5] A.W. Martinez *et al*., Anal. Chem., **82**, pp. 3-10, (2010).
[6] H. Hwang *et al*., Lab Chip, **11**, pp. 3404, (2011).
[7] N. Godino, *et al*. Proc. IEEE MEMS, Paris, France, pp. 1017, (2011).

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