RAPID POINT OF CARE (POC) BLOOD ANALYSIS USING INTEGRATED DYNAMIC BLOOD SEPARATION AND SANDWICH IMMUNOASSAY ON A POLYMER LAB CHIP

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ABSTRACT

This work demonstrates a seamless integration of dynamic blood separation with sandwich immunoassay on a chip for clinical diagnostics of serum analytes in fewer than 7 minutes. Human blood samples are loaded into the device and propelled by pulsatile pressure down a long channel to affect serum separation from cellular components. Serum samples are further analyzed for target analytes in a small serpentine channel with a volume less than 200 nL.

KEYWORDS: POC blood analysis, dynamic blood separation, immunoassay, polymer lab chip

INTRODUCTION

Existing commercial devices that integrate on-chip blood separation rely upon passive capillary force driven filters and integrated electrochemical immunoassays which usaully take longer than 15 minutes to obtain a result. Previous work demonstrated dynamic blood separation using pulsatile pressure driven flow of blood samples to effect separation into serum and cellular components [1] and elaborated on sandwich immunoassays in polymer microfluidics [2]. In this work we integrate dynamic separation with a high surface area to volume microfluidic optical sensor for rapid analyte detection on a polymer lab chip.

DESIGN AND FABRICATION

A human blood sample is injected into a long spiral channel and dynamic pressure is applied to drive the sample along the length of the channel (Fig1 (a)). At the end of the channel the separated blood sample passes a passive valved branch point that leads to a smaller serpentine channel where an immunoassay is performed (Fig1 (b)). Flow direction is reversed to direct the serum rich component of blood into serpentine microfluidic channel for an immunoassay (Fig1 (c)).

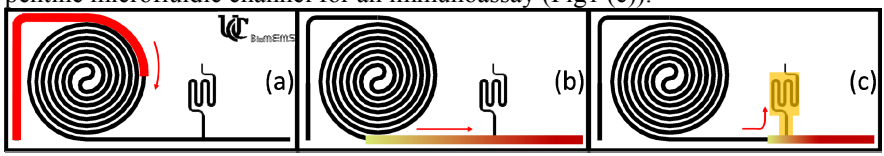

Figure 1. Schematic diagram outlining the function of the biochip with integrated dynamic separation and immunoassay: (a)Blood sample loaded into long channel; (b) Blood sample at the end of long channel using dynamic flow with a gradient of

978-0-9798064-1-4/μTAS2008/$20©2008CBMS

cells increasing from the back of the sample to the front; and (c) Serum rich component of the plug is flowed into a high surface area to volume serpentine sensor channel.

Dynamic separation is accomplished by protein blocking the channel, then flowing a small sacrificial sample of blood through the channel followed by a second sample (~250 nL) of blood pulsed through the channel. The theory behind channel pre-coating with a sacrificial blood plug is to eliminate forward and reverse capillary forces in hydrophilic channels by coating the channel with a sample of blood, equal and opposite capillary force in both forward and reverse directions when the second sample of blood is introduced to the channel (Fig 2).

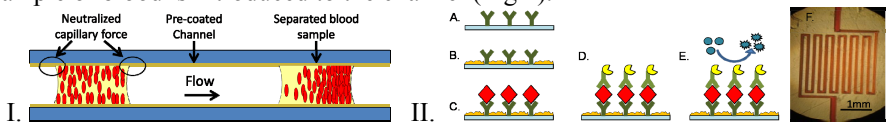

*Figure 2. **I)**Precoating the microfluidic channel with a small blood sample neutralizes capillary forces along channel corners and maintain sample as it is separated by pulsatile pressure and **II)** cTnT Immunoassay Steps: A) Immobilize primary capture antibody, B) Bare regions are blocked with protein blocking solution, C) Sample containing cTnT is flowed into channel, D) Peroxidase-conjugated 2^{o}Antibody is flowed into chamber, E) Peroxidase's substrate is added, light detected and amplified, and F) Photograph of high surface area to volume detection channel.*

Devices were fabricated in COC rigid polymer as described in [3]. Spiral channels were fabricated 150µm wide x 70 µm tall, 100 µm wide x 55 µm tall sensor channel isolated from dynamic separator spiral by a passive valve (Fig 3 (a)). Human Cardiac Troponin T (cTnT) was detected in cTnT-spiked human blood (AbCam) by chemiluminescent immunoassay (Fig 2-I (a)-(e)) in a high surface area to volume serpentine microfluidic channel (Fig 2-I (f)).

RESULTS AND DISCUSSION

Separation of human whole blood by dynamic separation shows a gradient of cellular separation with serum rich regions at the rear of the blood plug, and cell rich region at the front of the plug (Fig 3(b)).

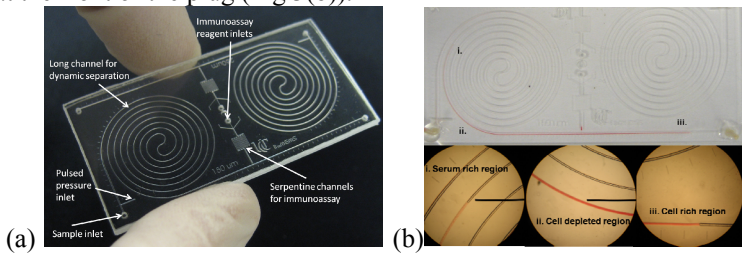

Figure 3. Blood separator: (a)Photograph of device fabricated in COC rigid polymer with two separation and detection fluidic circuits per chip and (b) Dynamic separation. Gradient of increasing cells is observed moving from the back of the sample(i) to the front of the sample (iii).

A calibration curve of cTnT immunoassay shows analyte detection extending down to 1.2 ng/mL from gravity separated human blood samples (Fig 4 (a)). Detection of 120 μg/mL cardiac Troponin T from serum samples separated by dynamic flow through a hydrophobic channel and separation by gravity confirms that channels that are not protein blocked result in target analyte depletion during separation (Fig 4 (b)).

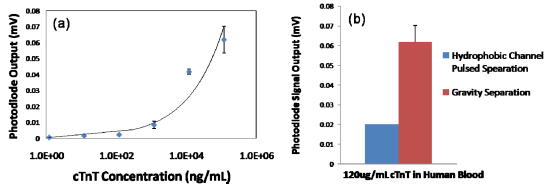

Figure 4. Experimental results: (a) Calibration curve for human whole blood spiked with increasing concentrations of cTnT and subsequent detection in serpentine microchanel by standard immunoassay and (b) Comparison of immunoassay for 120μg/mL human blood sample when blood is separated by pulsing the sample through an uncoated naked hydrophobic channel versus separation by gravity.

CONCLUSION

A polymer lab chip with dynamic blood separation integrated with a microfluidic immunosensing assay has been sucessfully developed and charatcterized for rapid point-of-care blood anlaysis in this work. Seamless integration of dynamic blood separation with on-chip sensors will enable blood analysis to be performed orders of magnitude more quickly than current devices relying upon capillary force driven serum separation.

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