PROTEIN QUANTITATION FROM WHOLE BLOOD ON POLYESTER-TONER LASER-PRINTED MICROFLUIDIC DISCS WITH CELL PHONE IMAGE ANALYSIS

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ABSTRACT

We demonstrate the use of polyester-toner, laser-printed microfluidic discs (PeT LPMDs) to provide simple and inexpensive methods for quantitatively determining clinical parameters from whole blood samples. Specifically, PeT LPMDs are utilized in combination with colorometric assays to create a quantitative clinical method for total protein, albumin, and hematocrit analysis. With full integration of multiple microfluidic functions, the work presented demonstrates a first generation PeT system capable of whole blood separation and component detection on one device. With the addition of cell phone analysis, as well as a simple rotational system, rapid and cost-effective clinical diagnostic testing can be achieved.

KEYWORDS: Polyester-Toner Laser-Printed Microfluidic Discs, Protein Quantitation, Cell phone analysis, Hematocrit

INTRODUCTION

Comprehensive chemistry panels are important for routine clinical diagnostic tests that help identify and quantitate a variety of parameters indicative of patient health status. Two important parameters are 'total protein' and 'albumin' concentration, the former serving as an important first indicator of multiple myeloma [1], while the latter is important as a nutritional marker, especially in dialysis patients [2]. The capability to obtain these quantitative protein measurements from small aliquots of blood in a rapid and cost-effective manner is critical for point-of-care applications.

Here, we utilize the continued advancement in microfluidic devices and technologies to develop a simple and inexpensive solution for clinical diagnostic measurements. In this work, three novel features for the colorimetric analysis of total protein and albumin are presented. The first is fabrication of a 5-layer polyester-toner laser printed microfluidic disc (PeT LPMD) (Fig. 1) that provides a simple, disposable, and cost-effective device made from easily accessible materials and fabricated with standard lab/office equipment [3,4]. With rotation-driven fluid flow, we show the capability for whole blood separation, plasma metering, and mixing through open microchannel architecture (Fig. 1) on a PeT LPMD. Second, the microdevice function is controlled by a portable and fully-automated system that utilizes embedded algorithms, which sequentially increase rotation speed in a temporal manner (Fig. 2). Finally, detection is colorimetric using a cell phone camera for optical imaging driven by a custom 'application' (app). Together, they demonstrate the potential to reduce time, expense, and expertise needed to perform simple clinical diagnostic testing that could be utilized in a point-of-care setting.

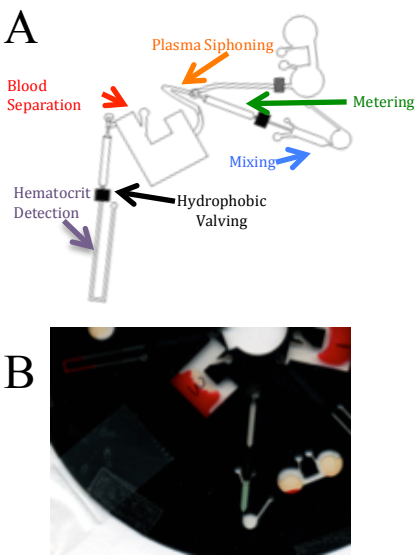

Figure 1: *(A) CorelDRAW design of protein colorimetric analysis PeT LPMD. Each function of the design is designated by a colored arrow. (B) Photograph of a LPMD after analysis.*

THEORY:

Here, whole blood is introduced into the separation reservoir, where the unique shape allows the hematocrit chamber to fill, while simultaneously keeping the sample from contacting the siphon valve. Upon centrifugation plasma comes into contact with the siphon valve. When rotation is stopped, the siphon is primed and rotation is reinitiated to fill the metering chamber. The plasma is held in the metering chamber through hydrophobic valves, in this case, patches of toner printed in the hydrophilic microchannels [5]. Fluid can only be forced through these valves when rotational speeds exceed the burst frequency of the hydrophobic patch, which can easily be altered by the amount of toner printed in the microchannel.

Figure 2: *Rotational control system in 3D printed case.*

Once the metering channel is filled excess plasma is redirected to a waste reservoir. The metered volume is then spun into the detection chamber, where it interacts with specific dyes. Mixing is achieved through the use of a unique surface tension pump that assists in the passive mixing of fluids present in the top and bottom layers of the LPMD. Due to the hydrophilicity of the transparency film, the capillary pressure generated in the detection chambers is greater than that in the mixing domain when the device is stagnant. Only when a rotation speed exceeds a specific value does the centrifugal pumping counteract the capillary pressure, driving the fluids to the mixing domain. It is here that micromixing occurs where the fluids are split and recombined repeatedly. A series of "spin-stop cycles" were then performed to ensure complete mixture of the fluids. Finally, image analysis was performed by quantifying 'hue' values associated with the resulting color change of the dye in response to protein concentration. Different colors can be detected by the camera and correspond to specific total protein and albumin concentrations. The image analysis application is then capable of graphing the collected data, where unknown protein concentrations can then be calculated.

EXPERIMENTAL:

Each PeT RDM is composed of five layers of transparency sheets, each of which is printed with toner and then laser ablated to achieve the desired fluidic architecture. The device fabrication is completed by thermally bonding the layers using an office laminator [3,4]. The total protein concentration is found with the use of tetrabromophenol blue (TBPB), while albumin concentrations are found with bromocresol green (BCG). Each assay is performed in approximately 10 minutes with colorimetric detection occurring at the end through a cell phone camera and custom image analysis app.

RESULTS AND DISCUSSION:

Initially, PeT LPMDs were utilized to mix the specific dyes with known total protein and albumin concentrations. Valid calibration curves that help distinguish protein concentrations through cell phone detection and analysis were then constructed (Fig. 3). Two calibration curves with excellent R^2 values (>

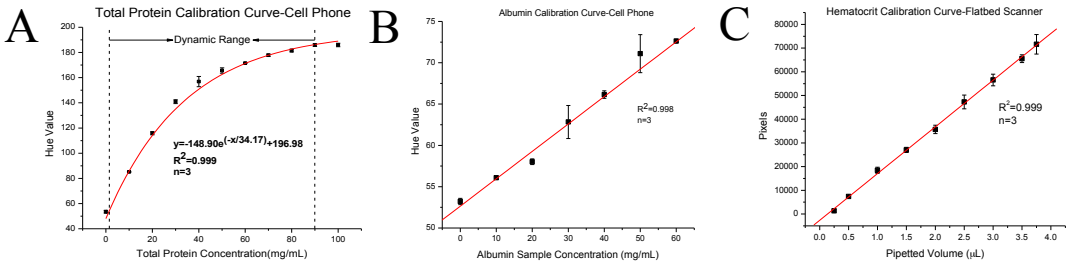

Figure 3: *(A) Total protein calibration curve by cell phone detection and analysis. (B) Albumin calibration curve by cell phone detection and analysis. (C) Hematocrit calibration curve by flatbed scanner and Wolfram Mathematica 8 image algorithm.*

0.997) are given in Figure 3A&B. In addition, a preliminary hematocrit calibration (R^2=0.999) performed with a flatbed scanner and image algorithm is given in Figure 3C.

Each calibration exhibits good dynamic range as well as excellent coefficients of determination and RSD values less than 5%. To prove the ability of the microdevice to perform protein quantitation and hematocrit analysis, blood samples that had previously been analyzed by conventional instrumentation were obtained from the clinical laboratory. PeT LPMD analysis of these samples using cell phone detection and analysis showed good correlation with clinical lab-validated total protein concentrations (Fig. 4A). In addition, after hematocrit analysis via a scanner and image algorithm, calculated percentages agreed well with values obtained from the clinical laboratory (Fig. 4B).

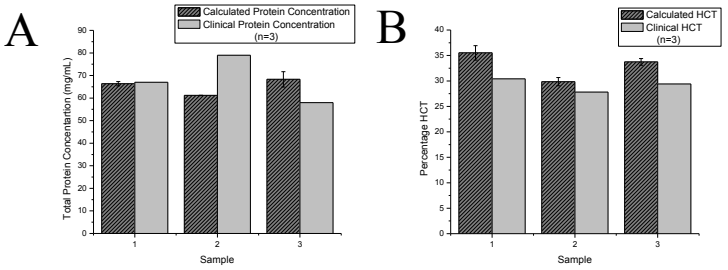

Figure 4: *(A) Calculated total protein concentrations compared to clinical values. (B) Calculated hematocrit percentages compared to clinical values.*

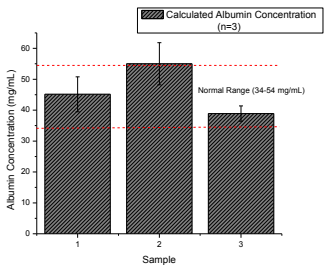

Figure 5: *Calculated albumin concentrations by cell phone detection and analysis.*

Finally, preliminary cell phone analysis of albumin concentrations can be seen below in Figure 5. Three blood samples using the cell phone detection showed three values in the normal range, indicating this method is capable of quantitating clinically relevant concentrations of albumin.

CONCLUSIONS:

Overall, a quantitative clinical assay has been demonstrated that integrates multiple microfluidic functions for protein quantitation and hematocrit analysis after blood separation is performed. We believe this is the first demonstration of colorimetric protein analysis of clinical value using PeT-based centrifugal microfluidics and cell phone image analysis.

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