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Hematocrit analysis through the use of an inexpensive centrifugal polyester-toner device with finger-to-chip blood loading capability

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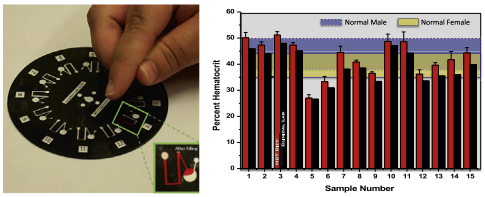
HIGHLIGHTS

- A 12-sample hematocrit device was developed from polyester-toner materials.
- The device can analyze a patient's hematocrit within 8 min from 3 μL of blood.
- Cell phone image analysis is used to correctly determine clinical hematocrits.

GRAPHICAL ABSTRACT



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ABSTRACT

Hematocrit (HCT) measurements are important clinical diagnostic variables that help physicians diagnose and treat various medical conditions, ailments, and diseases. In this work, we present the *HCT Disc*, a centrifugal microdevice fabricated by a Print, Cut and Laminate (PCL) method to generate a 12-sample HCT device from materials costing <0.5 USD (polyester and toner or PeT). Following introduction from a drop of blood (finger stick), whole blood metering and cell sedimentation are controlled by centrifugal force, only requiring a CD player motor as external hardware and, ultimately, a cell phone for detection. The sedimented volume from patient blood in the HCT Disc was analyzed using a conventional scanner/custom algorithm for analysis of the image to determine a hematocrit value, and these were compared to values generated in a clinical laboratory, which correlated well. To enhance portability and assure simplicity of the HCT measurement, values from image analysis by a cell phone using a custom application was compared to the scanner. Fifteen samples were analyzed with cell phone image analysis system and were found to be within 4% of the HCT values determined in the clinical lab. We demonstrate the feasibility of the PeT device for HCT measurement, and highlight its uniquely low cost (<0.5 USD), speed (sample-to-answer <8 min), multiplexability (12 samples), low volume whole blood requirement (<3 μL), rotation speeds (<4000 rpm) needed for effective measurement as well as the direct finger-to-chip sample loading capability.

1. Introduction

Microfluidics has garnered much interest since the introduction of the *miniaturized total analysis system* (μ-TAS) in the early 1990's [1]. Over the past 25 years, multiple applications in a variety of

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fields have emerged and have gathered strong interest that continues to grow as microfluidic technology advances. More specifically, microfluidic point-of-care (POC) systems have become a strong field of research in the past 10 years with an overarching goal of combining robust performance and effectiveness, ease of use by unskilled personnel, and cost-effectiveness. Microfluidics continues to be an attractive field for POC systems to reach this goal due to their small reagent and sample consumption, rapid analysis times, inexpensive nature, small footprint, and precise analyses.

A driving force in creating truly portable systems with minimized external hardware involves circumventing (or eliminating) the components needed for fluid flow control – e.g., pneumatic, hydraulic or syringe pumps. The use of centrifugal force for this purpose led to the development of *'lab-on-a-disc'* platforms [2] with numerous advantages that include portability and small footprint, while still providing the same functionality obtained in more standard microfluidic flow control approaches. Exploiting centrifugal forces, fluidic pumping, mixing, valving, cell sedimentation, volume metering, and flow switching are all possible, with many of these being integrated into multifunctional biomedical assays [3]. These include a variety of applications such as sample lysis [4–8], nucleic acid amplification [9–11], immunoassays [12–18], DNA microarrays [19–23] and colorimetric detection of biomarkers [24–26].

Here, we utilize rotation-driven microdevice technology as a concept for hematocrit (HCT) determination. Derived from *hemato* (from *haimat*, greek for *'blood*) and *crit* (from *krités*, greek for *'judge'*), the HCT is an assay that is used to measure the percentage of the total blood volume comprised of cells, predominately red (RBCs) and white blood cells (WBCs), following centrifugation [27]. The HCT is an important parameter in a panel of tests that are part of a 'complete blood count' (CBC), which is one of the more routine blood tests performed in medical diagnostics. As part of the CBC, the HCT is a central indicator of pathological conditions that affect the RBC count in whole blood. Low RBC levels can be indicative of RBC synthesis problems or RBC destruction (e.g., anemia), vitamin deficiencies, or traumatic blood loss that may result from internal bleeding. High HCT levels can signify dehydration, polycythemia, or lung and heart disease [27].

Traditionally, clinical laboratories perform the HCT analysis by the microhematocrit or Coulter counter method. Both are clinically-validated and used routinely to analyze patient blood samples for HCT. The microhematocrit method has a widespread popularity due to its simplicity and level of precision and accuracy [28]. While the long-standing use of the method in clinical labs is indicative of it effectiveness, it does not allow continuous measurements, and the high-speed centrifugal instrumentation can be expensive, large, and involve significant analysis times (>5 min), thus, providing significant challenges for adaptation to the POC environment. The other clinically-validated method uses a Coulter counter [29,30], which is a powerful instrument, unfortunately it is prohibitively large and too complex to allow for portability and use at the site of patient care.

In an attempt to bring HCT analysis to the POC, a number of microfluidic approaches have been developed to counter the limitations of the systems currently utilized in clinical chemistry labs. Riegger et al. demonstrated a centrifugal microfluidic disc that could be fabricated for ~$10 and could perform 9 HCT analyses simultaneously [31]. They described fluidic architectural advances including a novel bubble-free priming of a two-layer dead-end channel that was easily facilitated through capillary loading. Using centrifugal force, simple and inexpensive motors, and a commercially-available PC-CDROM drive, they were able to provide sufficient rotational speed for HCT measurement. While demonstrating POC potential, the disc fabrication protocol was complicated by surface coating and UV curing procedures that added process time to manufacturing and, thus, cost to the overall fabrication method.

Lee et al. developed a microfluidic device for HCT determination by measuring changes in the current under an applied voltage [32]. However simple, this impedance measurement approach required embedded electrodes, a power supply, as well as pumps for fluidic movement – external hardware that not only adds expense, but also decreases portability. Ironically, one of the most successful microfluidic commercial ventures, the i-STAT blood analyzer (Abbott Laboratories), uses impedance measurements to determine HCT and other important clinical parameters [33,34]. While extremely successful in terms of adoption by hospitals and clinics, it is expensive (~14,000 USD) with disposable cartridges costing 8-65 USD/cartridge, arguably expensive for POC testing.

Here, we describe the fabrication of a rotation-driven microdevice (RDM) using the simple, rapid and inexpensive PCL fabrication method [35], where the device cost can be < 0.50 USD, and potentially 0.05 USD with large-scale manufacturing. The microdevice can accommodate up to 12 samples, where spinning sediments the cells, and optical detection using either a scanner or a cell phone for image analysis allows for determination of the HCT value. Discriminating it from other approaches and highlighting the novelty of the device and assay, the microdevice design requires <3 μL of whole blood, which is advantageous for neonates or severely anemic patients, it avoids sample preparation steps and in a peerless manner, and provides a sample-to-answer time of <8 min, much more rapid than current approaches. Optical image analysis removes the potential for subjective error by the user, and the metering capabilities inherent in the microfluidic architecture decreases the error associated with sample handling, which can be found with other methods. Centrifugal force for fluid flow control circumvents the need for external pneumatic pumps, decreasing the size of the motor and overall system. These advantages make this specific device ideal for future implementation aimed at POC HCT measurements in a variety of operational environments where a simple and inexpensive method is required, e.g., resource limited or rural areas.

2. Results and discussion

After surveying the literature on microfluidic approaches for HCT measurement, we were able to define a number of metrics that should be met to enhance implementation. First, it was clear that the fabrication process needed to be simple and inexpensive – only then might it be possible to provide a multisample HCT system where the device cost to the customer would be 2$ USD. Second, the architectural design should be simple and meld effectively with the fabrication process, and the fluidics (loading, metering, sedimentation) should also be simple and, in a centrifugal platform, not require excessively high spin speeds. Third, detection should not be subjective (i.e., require visual interpretation), but optical and automated so that sample-to-answer can be achieved with minimal user intervention.

2.1. PCL fabrication

Fig. 1 displays the fluidic architecture for the *HCT Disc* as well as a schematic of the multiple layers associated with the final polyester-toner microdevice. Fig. 1A shows the final fabricated form of the *HCT disc* with exploded views of the section of the microfluidic device. The design exploits a simple structure that includes an inlet for sample loading, a U-shaped channel for cell sedimentation, an overflow metering arm to facilitate accurate blood volumes, and a waste chamber. Each of the three separate layers