ABSTRACT
This paper reports a novel centrifugal microfluidic device for integrated sample preparation of large-volume whole blood samples for nucleic acid analysis. We present a device capable of processing 2 milliliters of undiluted blood while still allowing for the integration with downstream amplification and detection steps. In biological validation experiments, this device was shown to yield separated plasma samples with high purity (> 99.99%) in less than half the time of gold standard hospital techniques. We further propose the need for heightened emphasis placed on the development of μTAS devices capable of interfacing with large-volume samples.

KEYWORDS: Centrifugal, Separation, Blood plasma, Nucleic acid analysis

INTRODUCTION
The separation of blood cells from plasma is an important first step in many bioanalytical diagnostics, as the cellular components of blood can inhibit proper PCR amplification and interfere with absorbance measurements. Therefore, in integrated micro-total analysis systems for blood-based diagnostics, blood cells must be separated from the plasma before downstream analysis steps can take place [1].

Many microfluidic systems have been developed to automate small-volume (<100 µL) blood separation using various separation mechanisms such as sedimentation, diffusion, microfiltering, dielectrophoresis and the Zweifach–Fung Effect to achieve a cell-free plasma sample [2][3][4]. However, relatively few microfluidic systems, particularly in centrifugal microfluidics, have been developed for blood sample sizes on the order of several milliliters to meet the limit-of-detection specifications of real-world samples, which may only contain very small concentrations of the target molecule (e.g., nucleic acid analysis of bacteria, viruses).

THEORY
Continuous flow systems are an attractive solution for processing large volumes within the framework of microfluidic devices, however, the published existing separation mechanisms are not well suited for the handling of high-hematocrit-level samples. A recently published overview of microfluidic plasma separation devices compared the achievable flow rates with several factors including initial hematocrit percentage and plasma purity[5]. Only the centrifuge-based and CD-based methods, both batch processes, were shown to separate raw blood samples at high purity levels. The non-batch microfluidic devices required hematocrit levels ranging from 3% to 25%; the raw blood sample required dilution with buffer, and therefore necessitating longer device runs and perhaps additional integrated enrichment or capturing processes.

Centrifugal or compact disc (CD)-based microfluidics offer a simple approach to blood sample preparation as they allow for the multiplexation, automation and miniaturization of the classical blood separation technique based on sedimentation. By exploiting density and size differences between the various blood components, one sediments the denser cellular components of blood and is left with a cell-free plasma sample. Several groups have demonstrated small-volume plasma separation of both raw and diluted blood samples using centrifugal microfluidic systems[6].

Figure 1: (Left) Exploded view of multi-layer fabricated centrifugal device. (Right) Close-up schematic of a single device on the disc, with explanations for each chamber and microchannel function.
EXPERIMENTAL

The large-volume centrifugal blood plasma separation device we present in this study consists of layers of plastic bonded via layers of double-sided pressure-sensitive adhesives (PSAs). The plastics fabrication process for multi-layer centrifugal devices was previously presented at μTAS 2009 [7]. An exploded view of the centrifugal device is shown in Figure 1 (left), with a close up schematic of a single device for plasma separation in the figure on the right. The procedure used in the validation of this device for blood cell sedimentation is as follows. In validation experiments, 2.0 milliliters of raw porcine blood (with anticoagulant K$_2$-EDTA) is first added to the sedimentation chamber through the sample inlet hole. The device is then securely attached to a Servo motor, and subjected to the pre-programmed angular velocities shown in Figure 2 (left). After cell sedimentation at 3800 RPM for 2.5, 5, or 10 minutes, the rotational speed is decreased to 350 RPM to allow the transfer siphon valve to prime, and the cell-free plasma can then be transferred to a separate collection chamber. Still images taken during this procedure are shown in Figure 2 (right).

To determine the plasma separation efficiency of the large-volume centrifugal device, raw blood samples were subjected to sedimentation runs of varying durations (from 2.5 minutes to 20 minutes) and their plasma purity was measured as the percentage of blood cells remaining in the collected plasma sample. Sedimentation efficiencies were compared for BD® PPT tubes and centrifugal-disc devices spun at equal relative centrifugal forces; all tests were performed in triplicate.

RESULTS AND DISCUSSION

The results from tube and CD-based device comparison experiments are shown in Figure 3. All sedimentation runs resulted in very high purity plasma samples, much greater than 99% purity, defined as the percentage of blood cells removed from the starting raw blood sample. In comparing the tube and CD devices for the five-minute sedimentation run, the CD-based devices resulted in separated plasma of higher purity. Specifically, sedimentation within the CD device for 2.5 minutes resulted in plasma of purity equivalent to plasma resulting from a tube device run of 5 minutes.

Plasma samples of purity 99.9% and above are all likely to respond similarly to analytical detection techniques in diagnostic devices. Although not shown in the graph, a tube sedimentation run of 2.5 minutes resulted in plasma purity levels of 58%, compared to 99.9997% purity from the CD device. This was an expected result, as the protocol for the commercial tube called for table-top centrifugation for at least 10 minutes. In situations where blood cell sedimentation needs to occur very rapidly, only the CD device would produce plasma with the required high purity levels.

The total process time including acceleration and deceleration times for the CD-based system is 320 seconds, much faster than a floor centrifuge, which requires a long deceleration phase so that the separated plasma is not disturbed from rapid deceleration of the rotor. This CD-based system automates the plasma collection into a separate chamber, removing the additional step of manual pipetting, thereby removing the need for this slow deceleration final step since the plasma is already separated into another chamber, and is no longer at risk for cell resuspension. Additionally, this design is able to be integrated with downstream processes (i.e. extraction, amplification, detection) within a single, automated centrifugal device.
CONCLUSION
This paper presents a centrifugal microfluidic device which was shown to separate large-volumes of blood plasma in high purity levels. This multi-layered plastic device demonstrated successful separation of plasma from 2 milliliters of whole blood in less than 2.5 minutes. While this example device demonstrated one possible method for dealing with large-volume samples, there remains challenges in the development of universal sample-driven devices. With heightened emphasis on large-volume sample integration, this sample-driven approach would enable saliva-, urine-, or blood-based nucleic acid analysis in an integrated µTAS device.

ACKNOWLEDGEMENTS
The authors gratefully acknowledge financial support for this research from the DARPA MF3 center at University of California, Irvine. We would also like to thank Dhivya Sridhar and Will Southard for their help in fabrication and testing of the devices.

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