Lab on a Chip

Mesoscale Blood Cell Sedimentation for Processing Millilitre Sample Volumes

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Mesoscale Blood Cell Sedimentation for Rapid Collection of Millilitre Samples†

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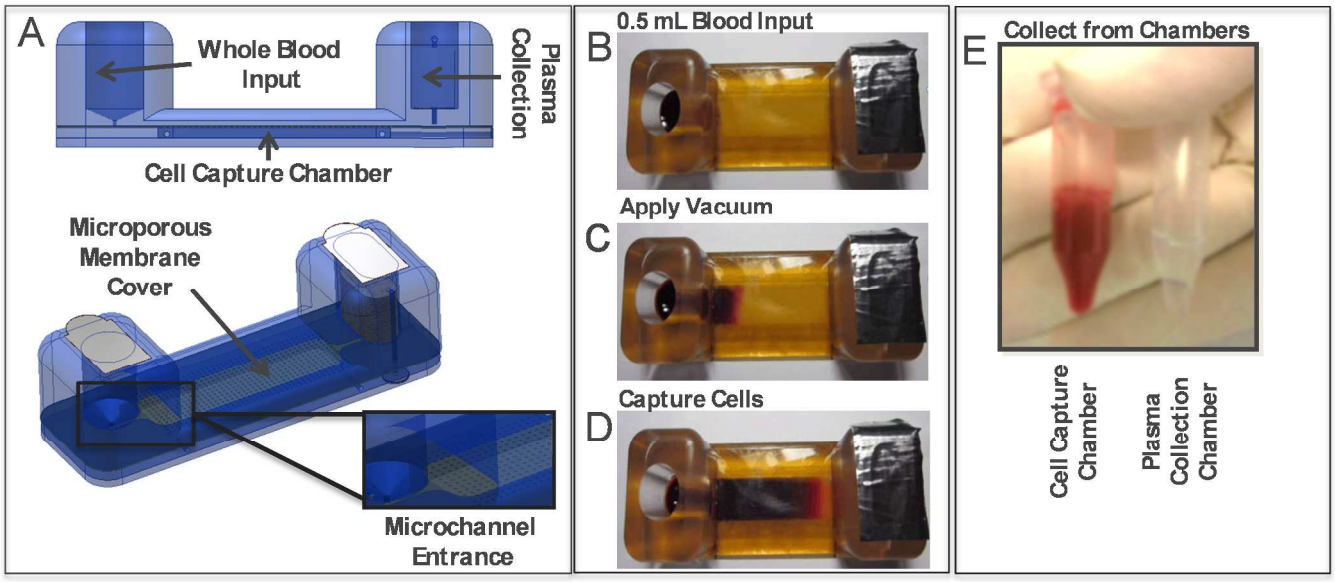

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We demonstrate the efficient separation of blood cells from millilitre volumes of whole blood in minutes using a simple gravity sedimentation device. Blood cell and plasma separation is often the initial step in clinical diagnostics, and reliable separation techniques have remained a major obstacle for the success of point-of-care or remote diagnostics. Unlike plasma collection devices that rely solely on microchannels that restrict sample volume and throughput, we demonstrate the use of a hybrid micro/mesoscale sedimentation chamber to enable >99% capture of cells from millilitre blood samples in less than two minutes.

Introduction

Rapid separation of blood into cell and cell-free components remains a critical need to enable remote sample collection and diagnostics, where access to a clinical centrifuge is not possible or a key contributing factor to pre-analytical variability. For example, isolating the cellular fraction from whole blood is generally the first step for analyzing genomic targets within peripheral blood cells, such as the *BCR-ABL* biomarker in chronic myeloid leukemia.[1] Likewise, obtaining cell-free plasma from whole blood is a critical need for measuring circulating nucleic acid (CNA) biomarkers[2-6]. However, rapid or remote fractionation of large volumes of blood samples is typically not feasible at the point-of-collection, and thus blood samples are typically stored and/or mailed until processing can be performed. Consequently, numerous pre-analytical variables can be introduced by delayed processing, including hemolysis and analyte degradation. In particular, the analysis of cell-free plasma is made challenging by nucleic acid degradation and white blood cell lysis (genomic DNA contamination) that occurs under standard blood storage and shipping techniques.[3-6] For these reasons, cold-storage, novel blood preservatives, and/or immediate purification are currently required to perform clinical assays, thereby limiting the economic feasibility of remote sample collection for centralized testing.[5,6] Additionally, PCR and sequencing based diagnostic assays that utilize cellular or cell-free NA often require millilitres of blood in order to accomplish adequate statistical biomarker sampling, and the cost required to transport these large sample volumes remains high.

Within the lab-on-a-chip community a number of techniques (i.e. gravitational sedimentation, inertial focusing, dielectrophoresis, and magnetophoresis) have been proposed for direct collection of cells and/or plasma from blood.[7-11] However, these microscale techniques remain ineffective in separation of components in large volume samples. Herein, we demonstrate the optimization of hydrodynamic entrance effects (i.e. microchannel and micropore cell stratification) for the efficient separation of blood cells into mesoscale sedimentation chambers. Optimization of the microfluidic features upstream of these larger chambers increases the blood volumes compatible with on-chip cell separation, and provides a technique compatible with equipment-free, point-of-care (POC) plasma separation. Compared to traditional microfluidic devices, plasma separation in our device is performed in whole blood at volume throughputs of > 0.5 mL/min. This simple and robust mesoscale blood cell separation device may be integrated with down-stream detection modules or used as a stand-alone device to eliminate the challenges associated with storage and shipment for remote sample collection.

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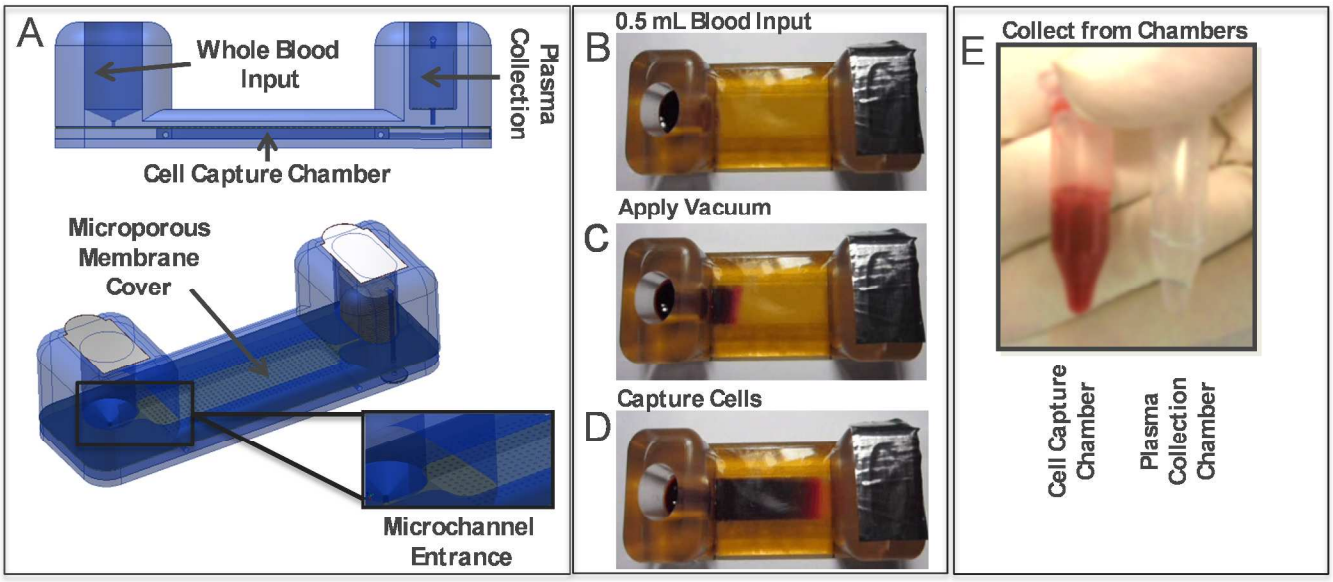

Figure 1. A. CAD drawing of the cell sedimentation device. The side view shows the three main compartments (whole blood input, cell capture/sedimentation, and plasma collection chambers). Insets show detail of the sedimentation chamber, including the microchannel entrance and microporous cover. B. Top view device images showing the three main steps involved in plasma collection (blood loading into the input chamber, vacuum applied to the plasma collection chamber, and cell sedimentation and capture). C. Example output from cell sedimentation and plasma collection chambers after running 0.5 mL whole blood through the device.

Experimental

The separation device shown in figure 1A was fabricated through adhesive lamination of rapid prototyped components (details in S1 ESI). The input chamber was designed to hold up to 1 mL input volumes of whole blood, while the plasma collection chamber was designed with 1.5 mL capacity to eliminate the potential for overfilling. The millimeter scale cell sedimentation chamber was connected to the input and collection chambers through two microchannels, and covered with a microporous membrane (medical grade polyamide mesh, 40 µm woven pores). The plasma chamber was sealed with metal foil, such that whole blood loaded into the input chamber could be drawn through the entire device by applying a vacuum to the collection chamber. The device was first primed with an isotonic buffer to maintain integrity of the captured blood cells during plasma collection; as a result the final concentration of plasma collected was diluted by the volume of buffer required to prime the device (details of plasma collection in S4 ESI). Figure 1B shows the loading of 0.5 mL of whole blood into the device and blood cell capture in the sedimentation chamber. Figure 1C shows an image of the liquid fractions collected from the sedimentation (cell containing fraction) and plasma collection chambers.

Blood cell quantification before and after plasma collection was performed using a Sysmex blood analyzer. During testing, an airtight syringe was attached to the vacuum port on the plasma collection chamber, and blood was drawn through the device at controlled speeds using a syringe pump. However, efficient blood separation was also accomplished using a simple, uncontrolled, hand-actuated syringe demonstrating high flexibility in device operation parameters (data from syringe operation in S3 ESI).

A finite element solution to the full Navier-Stokes equation was performed for the device using Comsol® multiphysics software. Velocity fields were extracted for multiple versions of the device with varying dimensions for the sedimentation chamber, the microchannel entrance to the sedimentation chamber, and for devices with or without the microporous membrane over the sedimentation chamber. The output boundary condition was set to 0 Pa, and inflow was set to 50 or 500 uL/min. Particle collection efficiencies were then modelled using the particle tracing function in Comsol, setting the particle/fluid density ratio and size to simulate white blood cells. These modelling results were used to determine final device dimensions (i.e. 2 mm deep sedimentation chamber, 100 µm input microchannel height, and 25 µm diameter micropores in the cover) for experimental verification of the cell and particle capture efficiencies (i.e. silica particles or white blood cells from whole blood).

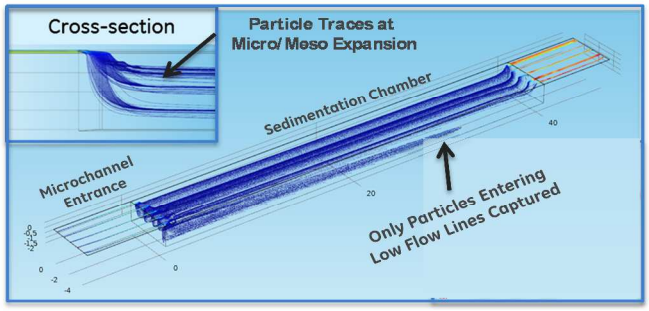

Figure 2. Results from the Comsol® CFD simulation demonstrating particle trajectories and capture within the sedimentation device (further details on capture efficiency in S2 ESI).

Results and Discussion

The Comsol® simulation particle trajectories shown in figure 2 demonstrate how the microchannel entrance to the sedimentation chamber acts to increase cell capture efficiency in the simple device. The three distinct particle paths shown within the sedimentation chamber were generated by "loading" particles at the center line of the upper third, middle third, and bottom third of the microchannel entrance. The model shows that there is an expansion of lamellar flow lines from the microchannel into the collection chamber. As a particle passes from the microchannel inlet to the collection chamber, it follows its respective lamellar flow line and the particle distribution is preserved as a function of relative channel height. Sedimentation of cells to lower flow lines within the microchannel acts as a high speed concentrator of cells into the lowest flow lines in the sedimentation chamber and decreases the transit times required to capture particles. Practically, this enables rapid cell stratification in the microchannel while maintaining large volume capacity in the sedimentation chamber, drastically increasing the cell capacity and throughput of the device compared to current microfluidic plasma separators. S2 ESI shows the results from initial trials using silica particles, which validate the model and clearly shows that capture efficiency is increased using a microchannel entrance to the sedimentation chamber. S2 ESI also shows that the addition of the microporous membrane cover also influences cell capture. While the microchannel concentrates particles in the lowest flow lines, the microporous membrane increases the total fraction of flow directed to the bottom portions of the sedimentation chamber and interferes with cell escape.

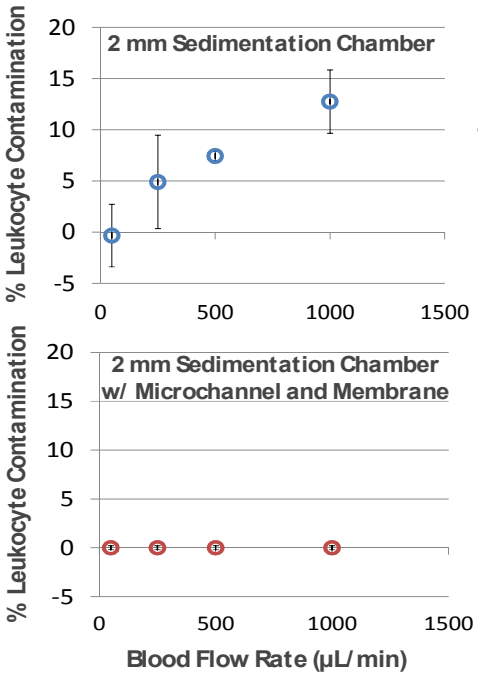

Figure 3. Leukocyte Contamination within the plasma collection chamber. Percent contaminating leukocytes using 0.5 mL whole blood input and mesoscale sedimentation chamber (2 mm) w/out (top) or with (bottom) the microchannel and microporous membrane features.

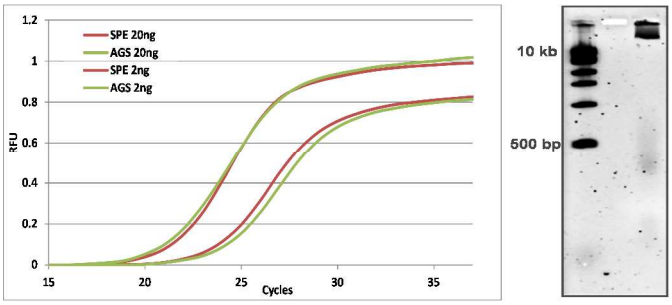

Figure 4. A. qPCR results using DNA extracted from cells collected using either standard centrifugation (red) or after sedimentation in the device (green). Ct values were equal in each condition at 20 and 2 ng of input DNA, confirming capture of the majority of cells within the sample using the device. In both cases DNA was purified using a commercial solid phase extraction kit after cell collection. B. Gel electrophoresis results from DNA collected within the plasma chamber showing a diffuse band with the majority of low molecular weight DNA below 500 bp.

Figure 3 demonstrates that within the 25 x 75 mm device footprint, the addition of the microchannel entrance and microporous membrane results in highly efficient blood cell capture at high volumetric flow rates. Figure 3A demonstrates that without the hybrid micro/mesoscale features (sedimentation chamber alone) white blood cell loss to the plasma collection chamber is high, reaching >10% cell contamination at rates of 1 mL/min. At 0.5 mL whole blood input this level of contamination equates to >250,000 cells and high levels of genomic DNA contamination within the captured plasma. Figure 3B demonstrates that with the addition of the microchannel and microporous membrane features, cell contamination within the plasma collection chamber decreased to < 1%. S3 ESI further demonstrates that the hybrid micro/mesoscale plasma collection device operates at much higher flow rates than current blood cell filters, while maintaining high cell capture efficiencies for down-stream analysis.

Figure 4A corroborates the Sysmex Blood cell counting data showing comparable qPCR results from DNA extracted from equivalent volumes of cells separated in the sedimentation device versus standard centrifugation. The PCR primers used were against a 380 bp amplicon heme target (FP–CTCACCCTGAAGTTCTCAGG, RP-GATGAAGTTGGTGG TGAGG). In addition, figure 4B shows a diffuse band of low molecular weight DNA captured from the plasma collection chamber (the majority of this DNA fell below 500 bp as expected in plasma).[12] However, figure 4B also shows a high molecular weight gDNA band, demonstrating some trace contamination of the plasma sample. A low level of contamination could be from a small number of cells that escape the sedimentation/capture chamber, but are not quantifiable using the Systmex analyzer at the low concentrations within the plasma chamber. The genomic DNA

may also be from damaged cells or cell fragments that escape capture within the sedimentation chamber. Future embodiments of the device will contain a final clean-up filter placed in front of the plasma collection chamber to further purify trace contaminants in the extracted sample, and to examine utility of the device in CNA diagnostic assays.

Conclusions

As healthcare cost pressures continue to drive investment in remote collection and at-home testing, there is an increasing need to provide simple and cost-effect methods of processing cells and plasma from whole blood. In addition, an increasing number of reports demonstrate that both processing and shipping/storage steps affect the down-stream diagnostic and prognostic potential of CNAs.[2-6] Recent demonstrations show that novel blood storage techniques may provide adequate NA stability for remote fetal mutation or chromosomal abnormality testing.[4] This data provides the first evidence that remote collection and analysis may be utilized to expand the geo-economic impact of CNA testing. However, further work is needed to decrease the cost and improve reproducibility during collection, shipping, and analysis of CNAs. In this report, we demonstrate a major step toward remote processing of high volumes of cells and plasma from blood in a simple to use device. We show removal of greater than 99% of cells from whole blood using microfluidic features at unprecedented flow rates (greater than 0.5 mL/min) using a hybrid micro/mesofluidic design.

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