DEVELOPMENT OF A MULTI-COMPARTMENT MICROFILTRATION DEVICE FOR PARTICLE FRACTIONATION

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ABSTRACT

Microfluidic cell separation and sorting techniques have been widely studied with relevance to cell biology research or various diagnostic and therapeutic applications. This work describes the development of a label free, low cost, high throughput device which uses cross flow filtration in order to fractionate particles based on size. The device is a multicompartment device with porous membranes of varying poresizes incorporated between the compartments within the microfluidic device to perform staged filtration where nonhomogenous cell mixtures are fractionated into different compartments and collected for further analysis. By varying the poresize between compartments, particles larger than the pores cannot pass through the membrane to the adjacent compartment and are selectivity enriched. This device was tested by infusing a mixture of polymer particles of varying sizes and a collected sheep's blood sample to examine size selectivity and particle recovery efficiency where white blood cells and platelets were selectivity enriched.

KEYWORDS

Microfluidic, Cell Separation, Hydrodynamics, Blood Analysis, Size Separation

INTRODUCTION

Cell separation devices are used in various biomedical applications to identify individual cell types within a mixed population, enrich a single cell type into subpopulations of similar types for downstream diagnostic analysis or therapeutic applications. Blood analyses, in particular, requires the separation of different components such as red blood cells, (RBCs), white blood cells, (WBCs), platelets and cell-free plasma for analysis or DNA purification from WBCs.

In this work, we present the development of a label free, high throughput, microdevice using a staged cross flow filtration module which fractionates particles based on size. The separation mechanism of the devices uses different pore size membranes incorporated between each compartment as physical barriers to fractionate particles from the sample specimen so that the size of particles which are to pass through to, or to are blocked from the next compartment is controlled and subpopulations can be collected for further analysis. The devices are designed to maximize filtration surface area with high level of cell presentation to the membrane pores.

DESIGN AND FABRICATION

The microfiltration device is fabricated with standard soft lithography techniques from polydimethylsiloxane (PDMS) microchannels bonded to semipermeable polycarbonate membranes coated with a solution of 3-aminopropyltriethoxysilane (APTES) [1]. This approach was previously used and developed to separate plasma from whole blood cells using a single membrane [2]. In order to create the multicompartment device, different pore sized membranes were aligned across the microfluidic structures in order to create the multicompartment structure. A prototype device containing 3 membrane pore sizes of 5 μm, 2 μm and 400 nm is schematically shown in Figure 1. The device is designed so that the lowest stringency filtration will occur at the left inlet reservoir with a progressively smaller pore sized membrane used in each compartment from the left to right through the device to increase the filtration stringency. Samples are infused through the left channel and the filtrates are recovered from 4 different outlets. Since particles are excluded from passing between layers if they are larger than the membrane pore size, cell mixtures can be depleted and fractionated by size into different compartments and samples can be collected from the outlets for further analysis.

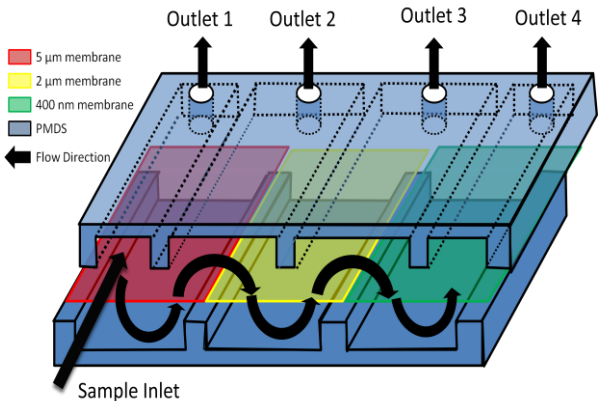

Figure 1. Schematic of the prototype device showing the individual compartments and flow path. The structure incorporates 5 μm, 2 μm and 400 nm poresized membranes to separate different compartments.

EXPERIMENTAL

A particle suspension containing 15 μm, 3 μm and 710 nm polymer fluorescent beads was infused through the device at 40 μL/min for 20 minutes and samples were collected at each outlet for particle counting. Next, whole sheep's blood (Hemostat Labs, Dixon, CA) containing a white blood cell population in a concentration of 6.34×10^7cells/mL was infused into the device at 60 μL/min during sample collection. The device was first primed with anticoagulant heparin (300 IU/L) at 10 μL/min for 10 minutes prior to blood infusion to prevent membrane clogging. The device was perfused from a 1 mL reservoir via a peristaltic pump (Model P720, Instech Laboratories, Plymouth Meeting, PA). Figure 2 shows

a photograph of the device during blood infusion. The blood was infused through the bottom left inlet channel. The first outlet was recycled back to the 1 mL blood reservoir to allow a continuous cell separation. An equal volume of the blood sample was added back to the reservoir in order to prevent depletion of the reservoir. Filtrate samples were collected in 10 minute fractions from each outlet and analyzed using a Coulter Counter (Model Z2, Beckman Coulter Inc, Brea, CA) to study the recovered cell populations for cell size distribution and concentration in each filtrate sample.

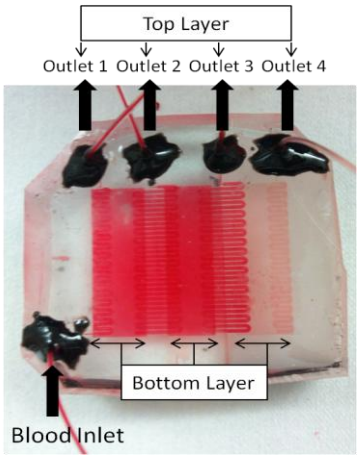

Figure 2. Photograph of the device while infusing sheep blood. The first outlet was recycled back to inlet reservoir to yield continuous cell separation. Both the top (serpentine channel structure) and bottom structures (wide channels) are labeled and are separated by the membranes.

RESULTS AND DISCUSSION

Figure 3 shows the particle selectivity of the device when a particle suspension containing 15 µm, 3 µm and 710 nm beads was infused through the device. No filtration occurs at outlet 1, so all three different sized particles were recovered (15 µm larger green, 3 µm smaller green and 710 nm small pink particles). At outlet 2 the solution was filtered across the 5 µm membrane and only the 3 µm and 710 nm particles were recovered. At outlet 3, the solution was filtered across the 2 µm membrane, and only 710 nm particles were recovered. Finally, at outlet 4 only fluid was recovered following filtration across the 400 nm membrane. After recovering the samples from each outlet, each particle concentration was counted and compared to their inlet concentration. Figure 4 quantifies the recovery efficiency of particles in the device where particles smaller than the membrane pore size were depleted from the originate compartment and recovered in a downstream compartment while larger particles cannot pass through the membrane barriers.

Recovery efficiency was characterized by normalizing each outlet concentrations to the original infusion sample concentration. At outlet 1, ~80% of the 15 µm particles, ~60% 3 µm particles and ~82% of 710 nm particles were recovered. At outlet 2, ~50% of 3 µm particles are recovered and ~60% 710 nm particles were recovered. At outlet 3, only the 710 nm particles were recovered with ~15% efficiency. At outlet 4, there were no particles and only fluid was recovered.

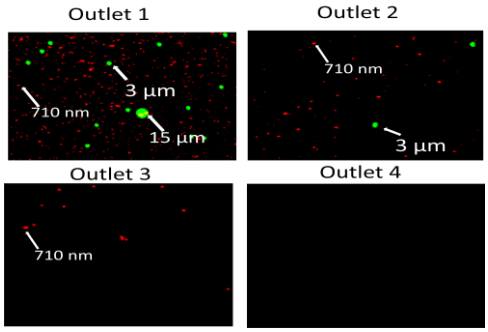

Figure 3. Micrographs showing selectivity of the device. Samples were collected and images were acquired using epifluorescent microscopy.

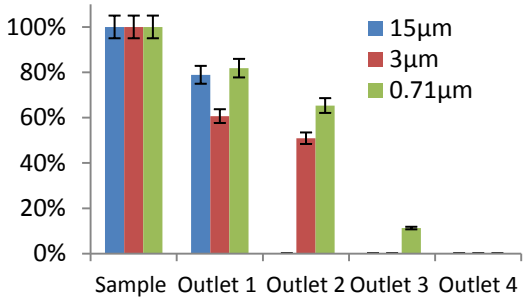

Figure 4. Recovery efficiency of the microfiltration device.

Next, the sheep's blood was perfused through the device as described above. The RBC concentrations collected at each outlet were examined (Figure 5). Due to the smaller size of the sheep red blood cell compared to human (~35 fL, 4-5 µm diameter for sheep and ~80 fL, 7-8 µm diameter for human), red blood cells can freely pass through the 5 µm membrane and were recovered at outlet 2. At outlet 3 which incorporated a 2 µm pore size membrane, the RBCs recovered are smaller than the mean of the whole blood population (mean volume ~30 fL). This is thought to be a result of the high perfusion flow rate and RBC deformability so that smaller cells squeeze through the membrane pores. Only plasma was recovered from outlet 4 which incorporated the 400 nm pore sized membrane with no cellular contamination.

The platelet and WBC concentrations recovered from the different outlets were also examined (Figure 5). Blood samples collected from each outlet were processed by lysing the RBCs using Zap-OGLOBIN II lytic agent (Beckman Coulter Inc, Brea, CA) for a more accurate platelet and WBC analysis. Platelets and white blood cells were recovered from outlet 1 because no filtration occurred. Since most of the WBCs cannot pass through the 5 µm membrane into outlet 2 only the smaller platelets were recovered from outlet 2 showing a further sub fractionation of the non-erythrocyte components of the blood. White blood cells were not detected at the downstream compartments at outlet 2, 3 and 4 showing size selectivity of the device. Platelets have volumes of less than 25 fL and therefore are small enough that they are depleted from the first compartment and recovered in the second compartment and were enriched at outlet 2 (Figure 6). Very few platelets were recovered from both outlet 3 and 4.

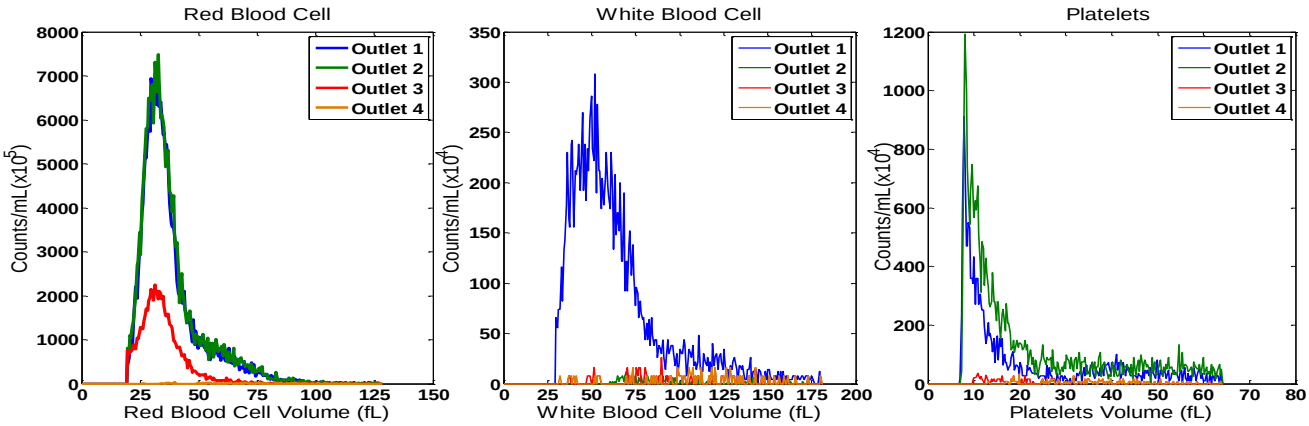

Figure 5. Components of sheep's blood recovered at device outlets after 10 minutes of device perfusion (Left) Red blood cell, (middle) white blood cell and (left) platelets counts analyzed via coulter counter at each outlet.

Figure 6 displays the platelet and white blood cell recovery and enrichment efficiency of the device. Samples were collected at three different time points, 10, 20 and 30 minutes after the blood perfusion started. All the samples were normalized to blood cell reservoir concentration when the experiment started. The white blood cell recovery efficiencies are greater than 100% because of recycling the samples from outlet 1 back to the reservoirs allowing WBC concentration to occur. After 10 minutes, ~100% of the white blood cells are recovered at outlet 1 and less than 1% of WBCs were detected at outlet 2 with no cells detected at outlet 3 or 4 indicating no contamination of non-erythrocytes within those 2 streams. At 20 minutes, ~210% of WBCs were recovered from outlet 1 which indicates the WBCs doubled their concentration due to enrichment with minimal cells detected at outlets 2, 3 and 4. After 30 minutes, ~280% of white blood cells were recovered from outlet 1, almost 3 times enrichment than the original WBC concentration without cell contamination at outlets 2, 3 and 4. From Figure 6 (right), after 10 minutes perfusion,~42% of platelets were recovered from outlet 1 and the remaining 56% of platelets were recovered from outlet 2 and ~2% recovered from outlet 3 with no cells detected at outlet 4. After 20 minutes, ~25% of platelets were recovered from outlet 1, ~70% of platelets recovered from outlet 2 and <1% recovered from outlet 3 and no cells detected at outlet 4. After 30 minutes, ~9% of platelets were left at outlet 1, ~90% of platelets recovered from outlet 2 and ~1% recovered from outlet 3 and again no cells at outlet 4. Thus, the smaller platelets were depleted from outlet 1 by passing through the 5 μm pore sized membrane and enriched at outlet 2. By recycling the blood sample from outlet 1, the device also shows the ability to enrich larger cells such as WBCs at outlet 1 while depleting smaller cells such as RBCs and platelets filtered into outlet 2 and cell free plasma can be obtained from outlet 4 demonstrating the ability to fractionate the blood based on cell size. Further experiments could incorporate different pore sized membranes to perform more selective fractionation (e.g., RBCs from platelets).

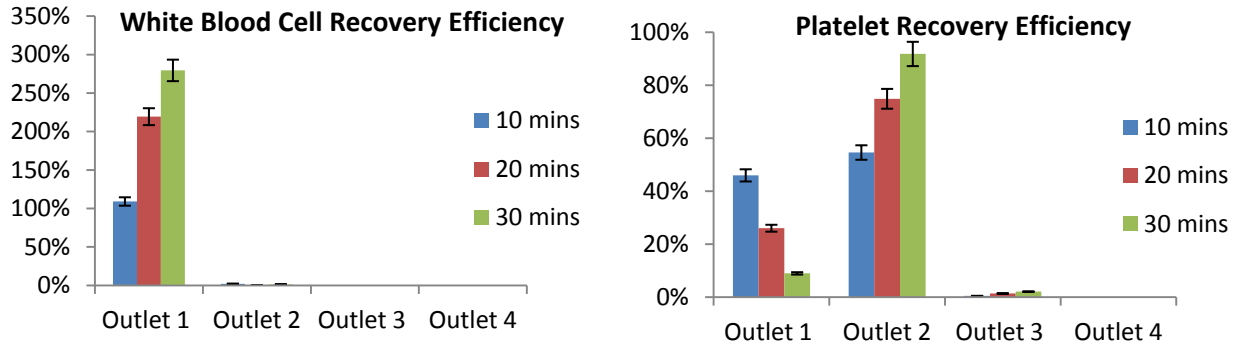

Figure 6. (Left)White blood cell recovery and (Right) Platelets cell recovery efficiency at different time points.

CONCLUSION

This work demonstrates the feasibility of fractionating differently sized particles via a staged filtration device which could be beneficial to various biomedical applications. This device can enrich different blood components such as platelets or white blood cells for clinical diagnostics**.** Future work will investigate the use of an actively controlled pneumatic pumping layer which could be incorporated with the device under the bottom microchannel layer to increase the filtration efficiency and to prevent membrane clogging by actively pushing cells out of pores.

REFERENCES

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