DEAN FLOW FRACTIONATION (DFF) ISOLATION OF CIRCULATING TUMOR CELLS (CTCs) FROM BLOOD

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ABSTRACT

Isolation and enumeration of circulating tumor cells (CTCs) as cancer biomarkers have been challenging due to their extremely low abundance in blood. This paper reports an ultra high-throughput technique for CTCs isolation from blood using the inherent Dean vortex flows present in curvilinear channels, aptly termed Dean Flow Fractionation (DFF). Using a 2-inlet 2-outlet spiral microchannel, the separation principle exploits the difference in cell size between CTCs (~16-20 µm diameter) and other blood cells (RBCs ~8 µm; leukocytes ~8-14 µm). Experimental results confirm >99% RBCs and leukocytes removal from blood sample (20% hematocrit) spiked with MCF-7 cells with >90% tumor cell recovery after separation. The developed technique offers large sample processing capability due to its ability to process very high hematocrit samples (20%), a key requirement for isolating rare-cells. A single device can process 1 mL whole blood in a single step, under 15 min.

KEYWORDS: inertial forces, spiral channels, Dean flows, circulating tumor cells

INTRODUCTION

Viable tumor-derived epithelial cells (or CTCs) produced during early stages of tumorigenesis have been identified in peripheral blood from patients with metastatic carcinomas and are responsible for extravasation at distant organs to form new metastatic sites [1]. As CTCs are extremely rare in blood (as few as one cell per 10^7-10^9 hematologic cells per milliliter) in patients with metastatic cancer, isolation and enumeration of CTCs as cancer biomarkers have been technically challenging. Many existing CTC isolation techniques rely on affinity-capturing of CTCs based on common cell surface antigens such as EpCAM [2]. However, widely accepted surface marker protein expressions are highly heterogeneous across different cancer types [3], thereby affecting the recovery rates of cancer cells. Inertial microfluidics based systems has recently been used for efficient cell separation, but one of the critical limitations is the requirement of relatively diluted blood for separation, thereby lowering overall throughput[4]. In this work, we introduce an ultra high-throughput technique for CTCs isolation from blood using the inherent Dean vortex flows present in curvilinear channels, aptly termed Dean Flow Fractionation (DFF). The method takes advantage of the size difference between the CTCs (~15-18 µm in diameter) and the other blood cells (RBCs ~8 µm; leukocytes ~8-12 µm).

DESIGN PRINCIPLE

The design consists of a 2-inlet 2-outlet spiral microchannel with 500×160 µm (w×h) dimensions. The spiral channel geometry gives rise to two-counter rotating vortices in the top and bottom half of the channel, known as Dean vortices, which apply a drag force on the cells [5]. Thus, cells flowing in a curvilinear channel experience a drag force due to the presence of these transverse Dean flows, entraining and driving them along the direction of flow within the vortices. This motion translates to the particles moving back and forth along the channel width between the inner and outer walls with increasing downstream distance when visualized from the top or bottom. Apart from the Dean drag force, larger cells with diameter comparable to the microchannel dimensions also experience appreciable inertial lift forces resulting in their focusing and equilibration. By selecting the appropriate channel dimensions such that the larger CTCs undergo inertial focusing, while the migration of the smaller hematologic cells (RBCs and leukocytes) is solely affected by the Dean drag, we can effectively isolate CTCs from blood [5]. By confining the sample to the outer half of the channel cross-section at the inlet and allowing one complete Dean cycle migration by choosing appropriate testing conditions (channel length, flow rate), CTCs are focused at the two equilibrium positions near the microchannel inner wall while the RBCs and leukocytes circulate along the Dean vortex and move back to the outer half of the channel (Figure 1)[5, 6]. Designing bifurcated outlets, the CTCs and hematologic cells can thus be fractionated and collected separately. The advantage of this technique is its ability to process very high hematocrit samples (~20%) thereby reducing sample preparatory steps and decreasing the overall analysis time as compared to microfluidics systems based solely on inertial forces [4].

EXPERIMENTAL PROCEDURES

The microfluidic device was fabricated in polydimethylsiloxane (PDMS) using standard soft-lithographic techniques and bonded irreversibly to microscopic glass slides using oxygen plasma treatment. The sample and sheath buffer were pumped using syringe pumps. Cell focusing and equilibration positions were experimentally observed using an inverted epi-fluorescence microscope equipped with a high speed camera (FASTCAM 1024 PCI).

Human breast adenocarcinoma cell line MCF-7 was used to mimic CTCs separation in this work. The cancer cells were cultured in low-glucose DMEM supplemented with 10% FBS together with 1% penicillin-streptomycin. Sub-confluent monolayers were dissociated with 0.01% trypsin and 5.3 mM EDTA solution. For device characterization, the cancer cells were diluted in buffer containing 1× phosphate buffered saline (PBS), 2 mM ethylenediaminetetraacetic acid (EDTA) supplemented with 0.5% bovine serum albumin (BSA) (Miltenyi Biotec, Germany) to prevent non-specific adsorption to the tubing and microchannel walls. For individual blood component experiments, blood obtained from healthy donors was adjusted to varying hematocrit (1–40%) with buffer and whole blood was treated with RBC lysis buffer (eBioscience, USA) according to the manufacturer's instructions to obtain a pure population of leukocytes. For whole blood analysis, blood samples were spiked with MCF-7 cells and diluted to 20% hematocrit (~2× dilution) for testing . Following separation, flow cytometry analysis using surface markers (EpCAM and CD45) was performed on the sorted samples to quantify CTC recovery from other hematologic cellular components.

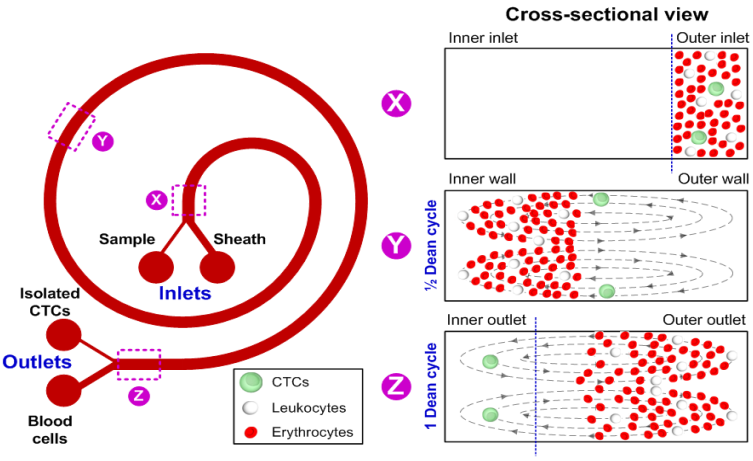


Figure 1: Schematic of the developed ultra-high throughput CTC isolation chip illustrating the operating principle. Blood sample is pumped through the outer inlet of the device while sheath fluid is passed through the inner inlet. Under the influence of Dean drag forces, the smaller hematologic cells (RBCs and leukocytes) migrate along the Dean vortices (cross-sectional view), while the larger CTCs experience strong inertial lift forces and focus along the microchannel inner wall, thus achieving separation.

RESULTS AND DISCUSSION

Figure 2 presents the experimental results for RBCs focusing positions with increasing hematocrit. After a single Dean cycle migration, the smaller RBCs were found at the outer half on the channel. Increasing the hematocrit results in the RBCs band broadening due to increased RBC-RBCs interactions. A final hematocrit of 20% was chosen as it allows collection of pure CTC fraction at the inner outlet. At 20% hematocrit, the cell free layer near the inner wall spans ~175 µm, thus completely eliminating RBCs from the CTCs outlet (150 µm).

Figure 3 illustrates the focusing positions of the MCF-7 cells and leukocytes at the channel outlet. The larger MCF-7 cells undergo inertial focusing and are equilibrated at the inner wall while the smaller leukocytes behave similar to RBCs and are transposed to the outer half of the channel. The outlet bifurcation was designed such that the CTCs outlet is 150 µm wide, thereby allowing efficient recovery (>90%) of the MCF-7 cells while ~99.99% RBCs and 99.6% leukocytes were successfully removed from the blood cell outlet (Figure 4).

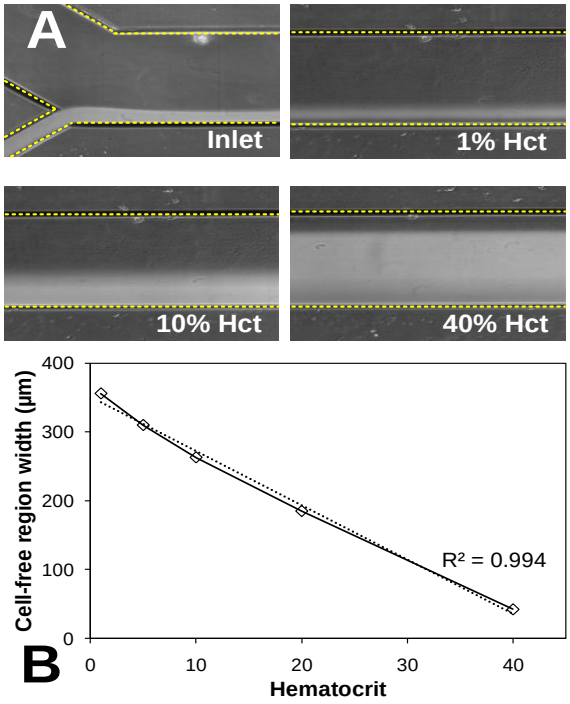


*Figure 2: (**A**) Averaged composite images illustrating the RBC occupied region for increasing hematocrit at the outlet. The sample to sheath buffer flowrate was fixed at 1:10. (dotted lines indicate position of channel walls) (**B**) Experimental result indicating a linear relationship between the width of the cell-free region from the inner channel wall and sample hematocrit.*

CONCLUSIONS

In this work, we introduce an ultra high-throughput technique for CTCs isolation from blood using the inherent Dean vortex flows present in curvilinear channels, aptly termed Dean Flow Fractionation (DFF). The DFF technique offers many advantages including continuous operating mode and the ability to process very high hematocrit samples (20%) thus decreasing the analysis time. A single device can process 1 mL whole blood in a single step, under 15 min. Large channel dimensions also eliminate clogging issues facilitating high repeatability. Lastly, the device dimensions can be easily customized for isolating other rare-cells from blood including leukocytes and fetal nucleated RBCs.

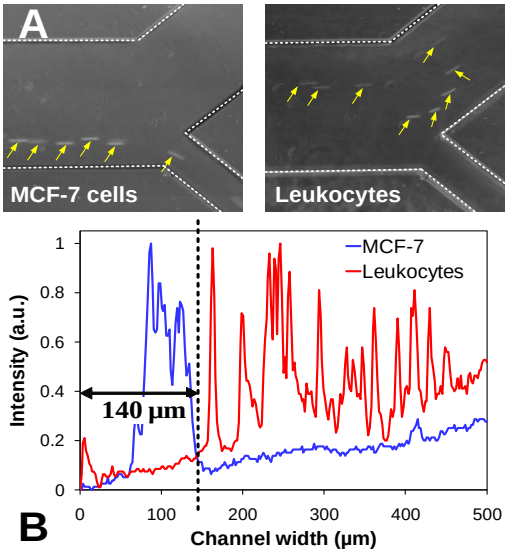
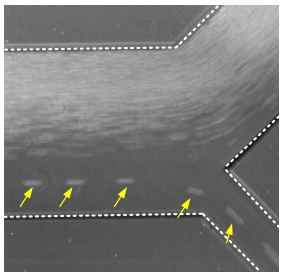


*Figure 3: (**A**) High speed images (6400 fps) and (**B**) linescans indicating the lateral positions of the MCF-7 cells and leukocytes at the outlet of the spiral sorter. The images clearly show the leukocytes are transposed to the outer half of the channel under the influence of Dean drag forces while the larger CTCs focus closer to the channel inner wall (<140 µm) under the influence of inertial lift forces.*



	Concentration (%)		
	RBCs	**Leukocytes**	**MCF-7**
Sample	100	100	100
CTC outlet	6.7E-3 ± 9.0E-4	0.38 ± 0.16	90.9 ± 11.0
Depletion	99.99%	99.62%	

Figure 4: (left) High speed image illustrating the fractionation and collection of MCF-7 cells (indicated by arrows) spiked into 20% hematocrit blood sample at the CTC outlet. (right) Table presenting experimental data of the relative cell concentrations recovered from the CTC outlet of the device measured by flow cytometry (FACS).

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