MICROFLUIDIC VORTEX TECHNOLOGY FOR PURE CIRCULATING TUMOR CELL CONCENTRATION FROM PATIENT BLOOD

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ABSTRACT

The isolation of circulating tumor cells (CTCs) from blood is important for determining cancer prognosis, characterizing genetic mutations for targeted drug therapies, and studying the biological mechanisms of metastasis. Here, we combine microfluidic inertial focusing and vortices to extract and enrich large CTCs from a significant background of smaller leukocytes. The size-based, clog-less technique operates at a high throughput (20 min for 7.5 mL of blood) and delivers a concentrated sample (300 µL volume) of cells at high purity (57-94%) and viability (85.7%). We demonstrate successful CTC extraction from stage IV cancer patients with breast (N=4) and lung (N=8) cancer.

KEYWORDS: inertial microfluidics, rare cell enrichment, cancer cell trapping, circulating tumor cells, vortex

INTRODUCTION

The enumeration of circulating tumor cells (CTCs) in blood is important in determining cancer prognosis and assessing treatment efficacy. CTCs are very rare - only 1 to 10 CTCs may be found among millions of white blood cells and billions of red blood cells in one milliliter of blood. Current approaches for CTC isolation, including affinity-based capture, size-based filtration, rapid optical scanning, and active forces (i.e., acoustophoresis or dielectrophoresis) each have unique advantages and disadvantages: these techniques are often limited by speed, the deformability of cells, variable gene expression for immunocapture, long sample preparation steps, cost, efficiency, and ability to deliver viable cells at high purity. Here, we make use of arrays of microscale laminar fluid vortices to quickly isolate CTCs from large volumes of blood at high purity and without labels. Microvortex trapping has unique advantages in achieving a low cost, high throughput, and high purity technique that yields freely available and viable cells.

THEORY

We make use of a microfluidic Vortex Chip which uses unique inertial fluid dynamic effects to passively isolate cancer cells from blood. It is a modification of previously described microvortex research [1] [2] with improved capture efficiency tuned for CTC extraction. The device is highly parallelized with an array of 8 straight channels (80 µm-tall × 40 µm-wide), each containing 8 rectangular reservoirs (480 µm-wide × 720 µm-long). Under high flow rates (4 mL/min), cells focus to equilibrium positions near the side walls of the straight channels (10 mm long), based on the balance of an inertial shear-gradient lift force and a counteracting wall-effect lift force (Figure 1B). Large cells (>15 µm), such as CTCs, experience a larger shear-gradient lift force and become trapped in laminar microvortices which form in the rectangular reservoirs (Figure 1C). Smaller cells (leukocytes and erythrocytes) do not experience sufficient lift force and pass through the reservoirs in the main flow. Cancer cell maintenance in microvortices allows for on-chip solution exchange to wash away any remaining blood cells. The captured cancer cells are released by lowering the flow rate to dissipate the vortices. The device rapidly enriches and releases a small volume (~300 µL) of viable cancer cells from blood at high concentration and purity.

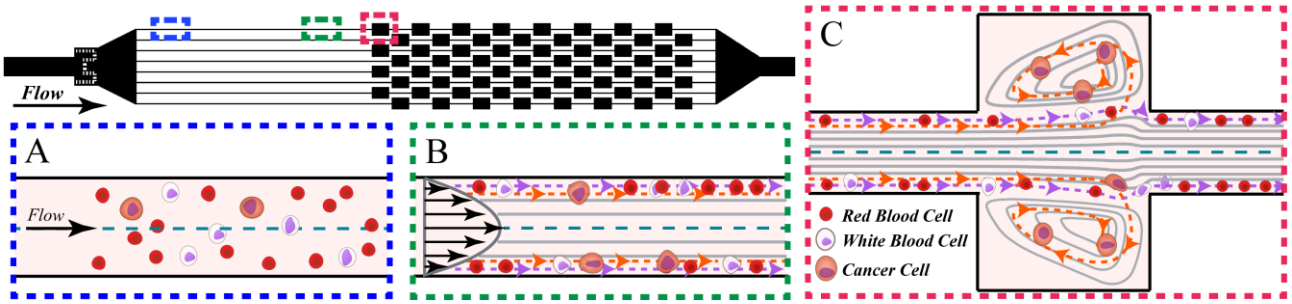

Figure 1: Vortex Chip design and function. (A) 10x diluted blood is directly infused into the device. Cells are initially randomly distributed. (B) Under high flow rate, cells focus toward the two lateral sides of a straight channel. (C) Larger cells (CTCs) experience a larger inertial shear-gradient lift force, allowing them to cross fluid streamlines and become trapped in a region of recirculating flow. Smaller cells (red and white blood cells) do not experience a sufficient lift force and remain in the main flow.

EXPERIMENTAL

Using a transparency photomask (CAD/Art Service, Inc.) and photoresist (KMPR 1050, MicroChem), standard lithographic techniques were used to fabricate a silicon wafer mold, over which polydimethylsiloxane (PDMS) was cured. PDMS and glass slides were activated by O_2 plasma before being bonded to form the device. The Vortex Chip was evaluated with melanoma (M395), ovarian (OVCAR5), breast (MCF7), lung (A549), and prostate (PC3) cancer cell lines spiked in healthy blood and 10x diluted with PBS buffer. The spiked samples were infused through the device at 4 mL/min using a syringe pump and released in a 96-well plate. Immunostaining was performed to enumerate cells for determination of capture efficiency, enrichment, and purity. Capture efficiency was quantified as the number of cancer cells released in the well plate over the total number of cancer cells injected through the chip. Purity was defined by the number of cancer cells over the total number of nucleated cells, and enrichment as the purity of sample after collection over the purity of sample before processing. A live/dead assay was performed using Calcein AM and ethidium homodimer, and cells were cultured over 4 days to determine viability and growth rates, respectively. Finally, blood samples from stage IV breast and non-small-cell lung cancer (NSCLC) patients were processed through the chip and enumerated by counting fluorescently labeled cells using cytokeratin-FITC (CTC), CD45-PE (leukocyte), and DAPI (nucleus) stains.

RESULTS AND DISCUSSION

The Vortex Chip successfully separates a variety of cancer cells from blood (~20% capture efficiency), including those from melanoma, ovarian, breast, lung, and prostate cancer cell lines, with on the order of 10,000 fold enrichment and >80% purity (Figure 2). Captured cells remained viable and could be cultured off-chip (Figure 3). Finally, tests with clinical blood samples demonstrate successful capture of CTCs from metastatic breast and lung cancer patients (Table 1). Blood from cancer patients exhibited higher CTC counts compared with those from healthy donors (Figure 4). Moreover, captured CTCs were characteristically larger than white blood cells and frequently multinucleated (Figure 4C).

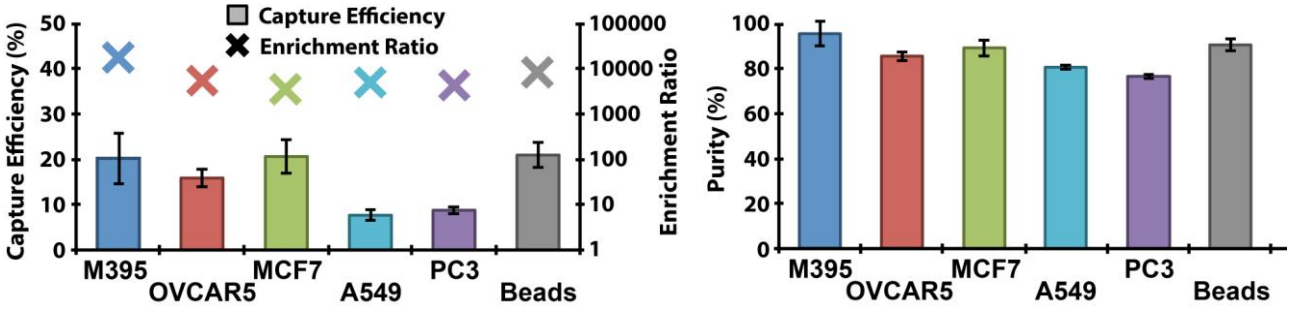

Figure 2: Vortex Chip performance with cancer cell lines spiked in blood. (A) The Vortex Chip achieves ~20% efficiency and exhibits up to a 10,000 fold enrichment ratio on a variety of cell lines. (B) After a solution exchange with an isotonic buffer, the flow rate is decreased and cells are released at purities >80%.

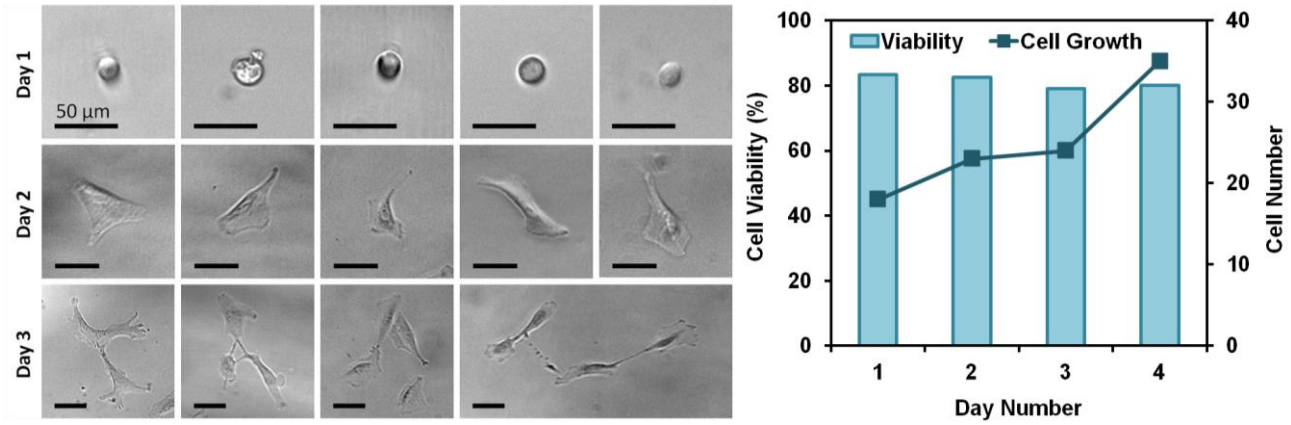

Figure 3: Cell culture and viability after release from the Vortex Chip. After processing through the device, A549 lung cancer cells become adherent and divide over the course of 4 days in a 96-well plate. Scale bars are 50 µm in the bright-field images. Cell viability is maintained at ~80%, and cell growth is observed over time.

Table 1. Summary of CTC enumeration results from cancer patients

Subjects	No. of Samples	Samples with > 5 CTCs/mL
Healthy	4	0%
Breast cancer	4	50%
Lung cancer	8	87.5%

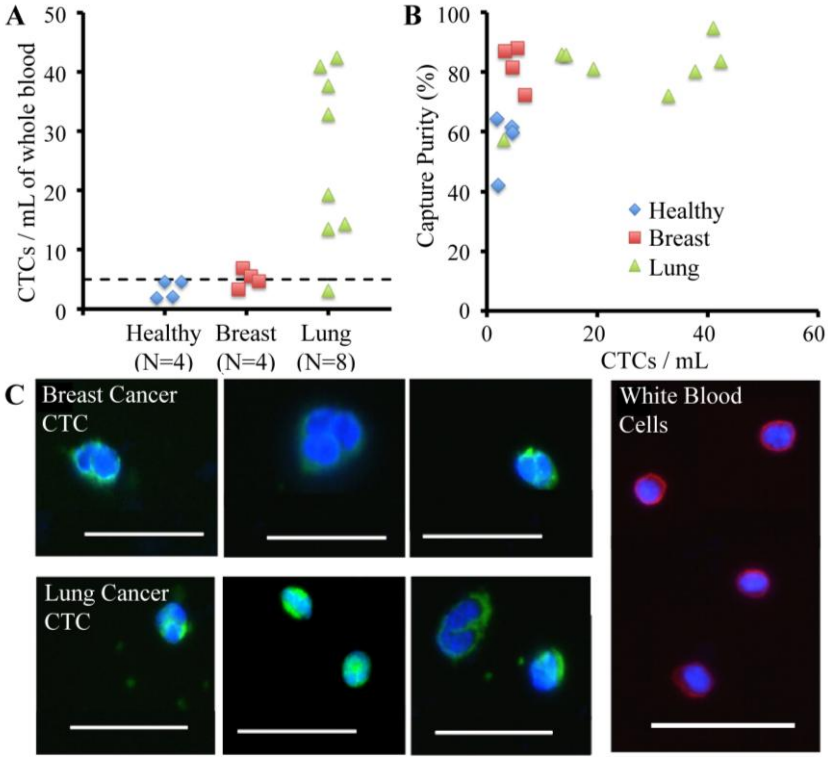

Figure 4: Capture of CTCs from stage IV cancer patients. Samples were counted to determine number of CTCs and white blood cells (WBCs) after processing with the Vortex Chip. (A) CTC counts were larger in cancer patients than in healthy blood donors. (B) The purity of CTCs was ~80% over all cancer patient samples. (C) CTCs (CK+/DAPI+, green/blue) appeared larger than leukocytes (CD45+/DAPI+, red/blue) in both breast and lung cancer samples. Multinucleated CTCs were frequently observed. Scale bar represents 37 μm.

CONCLUSION

The Vortex Chip's ability to extract pure, viable CTCs provides flexible opportunities for downstream applications, such as characterizing genetic mutations for targeted drug therapies and studying the biological mechanisms of metastasis. As a label-free, size-based isolation technique, the device may be applied to enumerate CTCs from prostate, colon, pancreatic, and other types of cancers as well. Similarly, the technology is applicable to other types of body fluids, such as urine, peritoneal, and pleural fluids, which are all minimally-invasive sources of rare cells with diagnostic value. Continued tests will be performed on clinical blood samples to gauge the correlation between CTC counts and patient outcome for a variety of cancers. Moreover, device performance will be compared with leading technologies on the market (e.g., the Veridex CellSearch system).

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