HIGH THROUGHPUT CIRCULATING TUMOR CELL ISOLATION USING TRAPEZOIDAL INERTIAL MICROFLUIDICS

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ABSTRACT

A novel Dean coupled inertial microfluidic device with trapezoidal cross-section spiral channel for ultra-fast, label-free enrichment of circulating tumor cells (CTCs) from clinically relevant blood volumes is reported in this work. Using this single spiral microchannel with one inlet and two outlets, we have successfully isolated and recovered more than 80% of cancer cell line cells spiked in 7.5 mL of blood within 8 min with high purity. Putative CTCs were detected and isolated from 100% patient samples with advanced stage metastatic breast and lung cancer using standard biomarkers. DNA fluorescence in-situ hybridization (FISH) was also carried out to evaluate HER2 status in CTCs isolated from patient samples.

KEYWORDS: Size Based Separation, Trapezoidal Cross-section, Circulating tumor cells, Inertial microfluidics

INTRODUCTION

The enumeration and characterization of circulating tumor cells (CTCs) isolated from blood samples of metastatic cancer patients provide a potentially transformative approach for cancer diagnostic and prognostic [1]. Since CTCs are extremely rare in patient blood, the isolation efficiency becomes a technical bottleneck of this application. An optimal technology for CTC isolation must aim to isolate the maximum number of viable tumor cells with acceptable degree of purity and minimum time and processing steps [2]. Recently, high-throughput passive particle sorting based on inertial migration of particle inside curvilinear microchannels has been reported [3, 4]. Inertial microfluidics devices exploiting the hydrodynamic forces for particle separation have distinct advantages over other CTC isolation methods. This methodology relies purely on microchannel dimensions, fluidic forces and particle size to achieve continuous, label-free, and clog-free separation with extremely high throughput and efficiency. In this paper, the performance of a trapezoidal cross-section spiral microfluidic channel for the isolation CTCs from clinical blood samples, and its associated downstream studies, are investigated (Fig. 1).

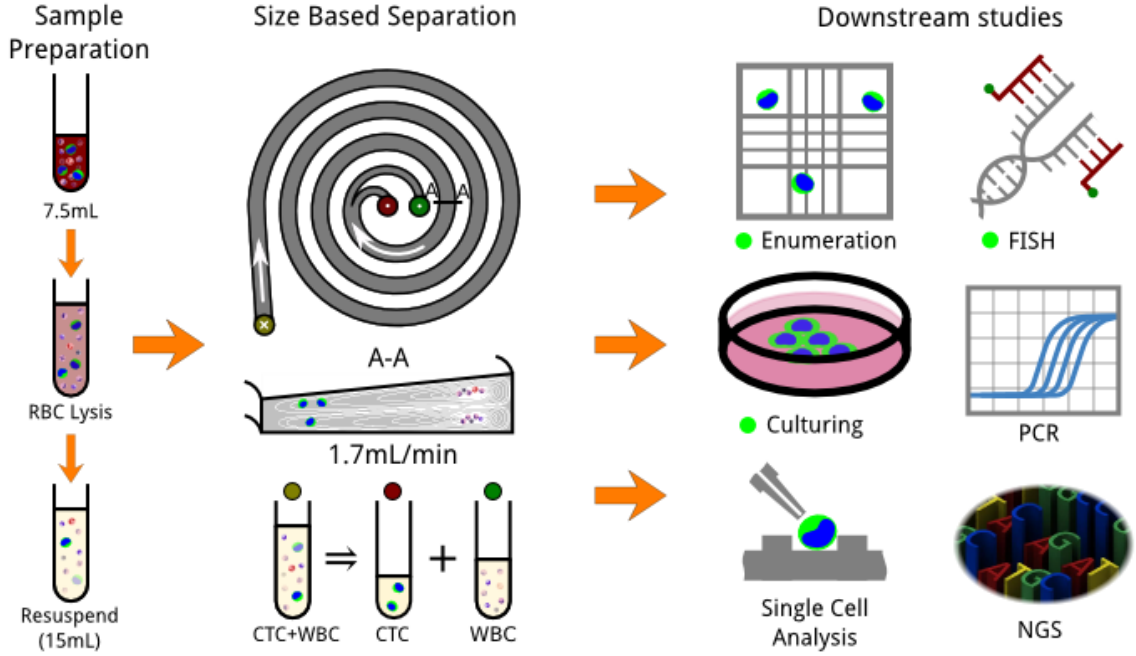

Figure 1: Process of CTC enrichment using the trapezoidal cross-section spiral microfluidic chip and its potential downstream assays (enumeration, culturing and FISH are introduced in this paper). The principle of separation is shown in the schematic of channel cross-section: CTCs are focused near the inner wall due to the combination of hydrodynamic forces while WBCs are trapped inside the core of dean vortex formed closer to the outer wall.

THEORY

When flowing through a curvilinear microchannel, particles suspended in a fluid experience inertial lift forces and Dean induced viscous drag. The combination of these forces will balance the particles at certain equilibrium positions of the channel cross-section in a continuous flow [3, 4]. Since both forces are a function of particle size, particles of different sizes occupy distinct lateral positions near the channel wall and exhibit different degrees of focusing, allowing size-based separation. One major challenge of utilizing a spiral microchannel with rectangular cross-section is the relatively small spacing between equilibrium positions of particles with varying diameters, which limits the separation resolution and throughput. Therefore, in contrast to the rectangular cross-section channels, where small particles focus near the center of channel width, the modified velocity field of trapezoidal cross-section spiral channels create strong Dean vortex cores near the outer wall that trap smaller particles, while larger particles remain focused near the inner channel wall (Fig. 1). As a result, the gap between these two cell streams will be maximized, leading to the enhanced separation throughput and efficiency [4, 5].

EXPERIMENTAL

An 1-inlet-2-outlet PDMS microfluidic chip with a trapezoidal cross-section spiral channel (having a radius of curvature from 7.5mm to 12.5 mm, 1mm pitch) was cast from a conventional micro-milling PMMA mold (Whits Technologies, Singapore) following standard PDMS casting process (Fig. 2A). The chip was calibrated with size standard micro particles (10µm and 15µmm, Bangs Laboratories, Inc. USA) to achieve the optimized flow rate and concentration (Fig. 2C and 2D). Blood samples from healthy donors and three cell lines (i.e., MCF-7, T24 and MDA-MB-231) were used to quantify the performance of the trapezoid spiral biochip for CTC isolation and recovery.

Human whole blood samples were obtained from healthy donors and metastatic lung and breast cancer patients. A total of 5 blood samples from healthy donors were used as controls and 10 samples from both lung and breast cancer patients were processed for CTC enumeration. Blood samples were collected in vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ, USA) containing EDTA anticoagulant and were processed within 2-4 h to prevent blood coagulation. To increase the purity and minimize the cellular components passing through the spiral chip, for all the samples, 7.5 mL of whole blood was lysed initially using RBC lysis buffer (G-Biosciences, USA) and re-suspended in 1×PBS prior to processing (Fig. 1).

Fluorescence in situ hybridization (FISH) was performed on SKBR3 (amplified HER2 signals) and MDA-MB-231 (non-amplified HER2 signal) cells lines as well as isolated patients' CTCs according to the manufacturer's protocol.

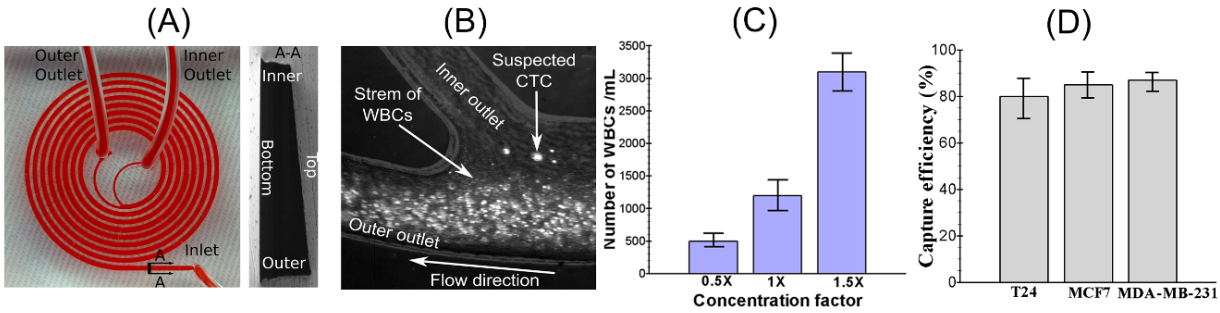

Figure 2: (A) An actual red-ink-dyed spiral chip with a microscopy image of the cut view of the trapezoidal cross-section channel (80/130µm ID/OD, 600µm wide). (B) Microscopy image of a breast cancer clinical blood sample processing near the outlet bifurcation of the chip (with cells image enhanced by ImageJ). (C) Number of WBC remains in the inner outlet collection of the chip at various sample concentrations. (D) Recovery efficiency of different cell lines.

RESULTS AND DISCUSSION

The optimum sample concentration and operation flow rate were 0.5× (re-suspend the RBC lysed sample with 1×PBS of 2 times of the original blood volume) and 1700 mL/min, respectively (Fig. 2C). This translates to a total processing time of 9 min for a 7.5 mL blood sample using a single chip. Under this flow rate, the total number of WBC in the recovery stream (inner outlet) is ~500 cells/mL. An average capture efficiency of 80% for T24, 85% for MCF-7 and 87% for MDA-MB-231 cell lines (n=3) was found, respectively (Fig. 2D). The cells were re-cultured onto 2-D culture substrates under standard culture conditions after the separation. The attachment and proliferation indicates that the cells retain integrity and remain healthy under the shear flow through the chip.

Presence of isolated patients' CTCs was determined by immunostaining with Hoechst (DNA), FITC-pan-cytokeratin (CK) antibodies (cancer/epithelial biomarker), and APC-anti-CD45 antibodies (hematologic biomarker) (Fig. 3). Hoechst+/pan-CK+/CD45- cells were scored as CTCs. Putative CTCs were detected and isolated from 100% (10/10) patients samples with advanced stage metastatic breast and lung cancer using standard biomarkers with frequency ranging from 6 to 57 CTCs/mL for MBC samples and 3 to 125 CTCs/mL for NSCLC samples (Fig. 4A). Epithelial cells positive for cytokeratin were detected in healthy volunteers (1-4 per mL), but a distinct detection threshold of 3-4 CTC per 7.5 mL can be drawn in comparison to that of patient samples for predicting metastatic disease. Viable cells were proved to be able to spread onto the substrates and appear negative for Propidium iodide (PI) when stained.

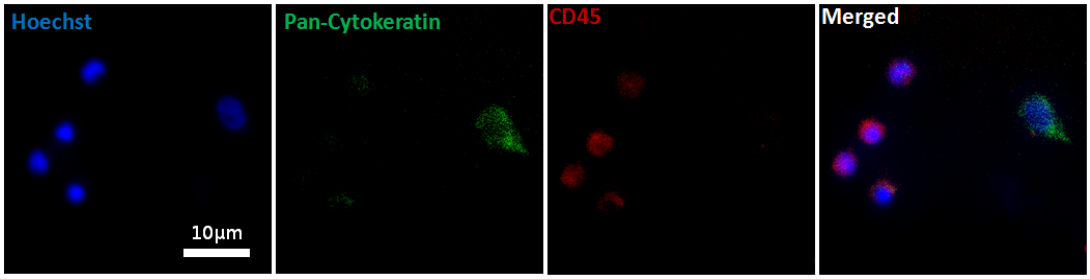

Figure 3: Immunofluorescence staining of isolated CTC (the one presents at the right side of the images) as well as remaining WBCs (the rest 4 at the left side of the images) from a lung cancer patient blood with the trapezoidal cross-section spiral chip. The CTC is identified by the following criteria: Hoechst+, pan-cytokeratin+ and CD45-.

HER2 status in CTCs varies with respect to the primary tumor. Specifically, HER2+ CTCs may be observed in ~30% of samples obtained from HER2- origin [6]. The comparison of HER2 signals in isolated CTCs against control breast cancer cell lines MDA-MB-231 (non-amplified HER2 signal) and SKBR3 (amplified HER2 signals) is shown in Fig. 4B. Amplified HER2 expression is determined when the ratio of HER2/centromere of Chromosome 17 (Cen17) signals in single nuclei is greater than 2. A range of HER2/Cen17 signal was observed. Cells displaying a ratio of HER2/Cen17 =1 are likely to be WBC or non-amplified HER2 CTCs, and can be distinguished with further immunostaining. However, cells with amplified HER2 signals were also detected, indicating the definite presence of CTCs. This is in accordance with previous findings that heterogeneity of HER2 status is evident in CTC as compared to the primary tumor [6].

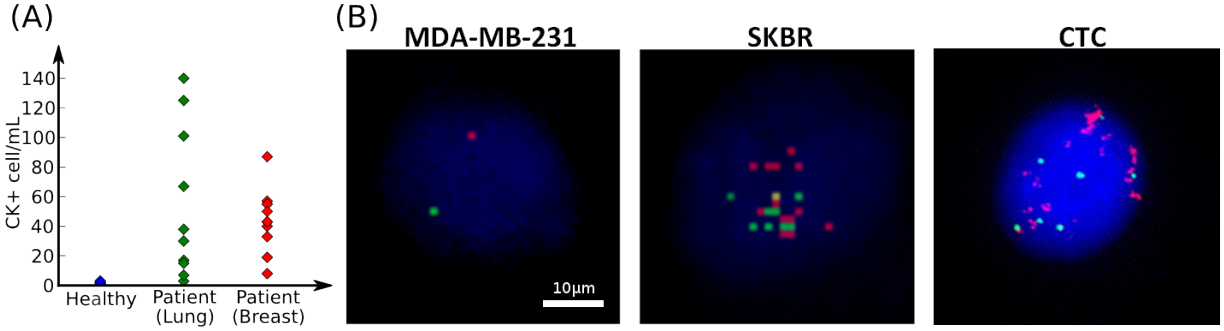

Figure 4: (A) Enumeration of CTCs isolated from healthy donors (n=5) as well as patients with metastatic breast and lung cancer (n=10) with the chip. (B) Merged images (20×, DAPI, Spectrum orange: HER2 signal, Spectrum green: Cen17) shows the detection of Cen17 and HER2 of enriched CTCs isolated from breast cancer patient. Cells were amplified for HER2 if HER2/Cen17 >2. MDA-MB-231 and SKBR3 breast cancer lines were used as controls.

CONCLUSION

The trapezoidal cross-section spiral chip can be produced with extremely low-cost using conventional micro-milling and PDMS casting, and can be operated without any pretreatment and/or antibody immobilization. Due to its throughput and efficiency, a host of downstream studies can be enabled for the examination of clinical blood samples for CTCs via the inertial microfluidic system described here. We strongly believe that this novel strategy can be utilized for large-scale processing of clinical samples in order to enrich sufficient amount of CTCs for various molecular analyses as well as clinical monitoring of individual patients undergoing therapy. The device is well suited to process even larger quantities of blood if required to meet a growing need for obtaining large number of CTCs for multiple downstream tests.

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