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Short communication

Versatile label free biochip for the detection of circulating tumor cells from peripheral blood in cancer patients

Swee Jin Tan[a,b], Rumkumar Lalitha Lakshmi[c], Pengfei Chen[c], Wan-Teck Lim[d], Levent Yobas[b,e], Chwee Teck Lim[a,c,f,*]

[a] NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, 12 Medical Drive, Singapore 117598, Singapore
[b] Institute of Microelectronics, A*STAR (Agency for Science, Technology and Research), 11 Science Park Road, Singapore 117685, Singapore
[c] Division of Bioengineering and Department of Mechanical Engineering, 9 Engineering Drive 1, Singapore 117576, Singapore
[d] Department of Medical Oncology, National Cancer Centre Singapore, 11 Hospital Drive, Singapore 169610, Singapore
[e] Department of Electronic and Computer Engineering, Hong Kong University of Science and Technology, Kowloon, Hong Kong
[f] Mechanobiology Institute, T-Lab 5A Engineering Drive 1, Singapore

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ABSTRACT

The isolation of circulating tumor cells (CTCs) using microfluidics is attractive as the flow conditions can be accurately manipulated to achieve an efficient separation. CTCs are rare events within the peripheral blood of metastatic cancer patients which makes them hard to detect. The presence of CTCs is likely to indicate the severity of the disease and increasing evidences show its use for prognostic and treatment monitoring purposes. We demonstrated an effective separation using a microfluidic device to utilize the unique differences in size and deformability of cancer cells to blood cells. Using physical structures placed in the path of blood specimens in a microchannel, CTCs which are generally larger and stiffer are retained while most blood constituents are removed. The placements of the structures are optimized by computational analysis to enhance the isolation efficiency. With blood specimens from metastatic lung cancer patients, we confirmed the successful detection of CTCs. The operations for processing blood are straightforward and permit multiplexing of the microdevices to concurrently work with different samples. The microfluidic device is optically transparent which makes it simple to be integrated to existing laboratory microscopes and immunofluorescence staining can be done in situ to distinguish cancer cells from hematopoietic cells. This also minimizes the use of expensive staining reagents, given the small size of the microdevice. Identification of CTCs will aid in the detection of malignancy and disease stage as well as understanding the phenotypic and genotypic expressions of cancer cells.

1. Introduction

Cancer is a leading cause of death, and in most cases, cancer deaths are the result of metastasis (Steeg, 2006) with malignant cancer cells spreading to distant sites. The lack of early warning at initial stages of the disease limits the effectiveness for cancer treatment (Chambers et al., 2002). Circulating tumor cells (CTCs) are disseminated from solid tumors that enter the blood circulation during hematogenous metastasis (Poste and Fidler, 1980) and recent clinical studies show that the quantity of CTCs in circulation is a good measure for prognosis and overall survival (de Bono et al., 2008; Pantel and Riethdorf, 2009; Slade and Coombes, 2007). It is also reported to have association to disease progression (Cohen et al., 2009; Cristofanilli et al., 2004) and treatment efficacy (Reuben et al., 2008; Urtishak et al., 2008). Hence, the blood specimens of cancer patients are a potential source of tumor cells. Furthermore, blood extraction is routinely performed for various health tests and also less invasive compared to surgical biopsies. The availability of blood samples is attractive for CTC enumeration to complement current techniques in the detection and monitoring of cancer.

The technical challenge to detect CTCs in peripheral blood lies in the rarity of these cells (Zieglschmid et al., 2005). The cell count can be as low as 1 cancer cell to 1 ml of blood which contains approximately 4.8–5.4 billion erythrocytes; 7.4 million leukocytes and 280 million thrombocytes (Fournier, 1998). Leading technologies in CTC enrichment from blood specimen uses affinity based techniques which employ antibodies that are expressed only on cancer cells (Cohen et al., 2008; Riethdorf et al., 2007). These methods face various drawbacks such as the need for pre-sample preparation and more importantly the specificity of the antibody selected for enrichment. Additional preparatory steps are likely to incur CTC losses while the lack of a universal biomarker for CTC enrichment limits

* Corresponding author at: Nano Biomechanics Laboratory, Division of Bioengineering, National University of Singapore, 2 Engineering Drive 3, E3-05-16, Singapore 117576, Singapore. Tel.: +65 6516 7801; fax: +65 6773 2205.
E-mail address: ctlim@nus.edu.sg (C.T. Lim).

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the detection of CTCs for different cancer types (Allard et al., 2004; Sieuwerts et al., 2009). The technique is also laborious, complicated and potential important information about the subpopulations of these cells may also be lost. Furthermore, the isolated cells may no longer be viable after processing of the blood samples which limits the downstream applications that can be done on CTC subpopulations. These tumor cells hold important information to the metastatic process and will aid in understanding the disease better (Pantel et al., 2008; Slade and Coombes, 2007).

Using a microfluidic device, we aim to achieve an effective CTCs separation from peripheral blood using distinctive physical differences between cancer cells (Weiss, 1990; Weiss and Dimitrov, 1986) and blood constituents (Mohamed et al., 2004; Shelby et al., 2003). We had previously reported on the use of the microdevice in the isolation of breast and colorectal cancer cell lines and optimized the design with computational fluid dynamic simulations (Tan et al., 2009). In the current study, we extended the investigation to cover a wider range of cancer types to determine the versatility of the technique to handle different cancers as the disease is genetically heterogeneous (Braun et al., 1999; Reya et al., 2001; Shah et al., 2004). In addition, we demonstrated the sensitivity of the system with the successful recovery of low counts of cancer cells in numerous tested samples. From the peripheral blood of metastatic lung cancer patients, the platform is tested to detect CTCs and characterized for the isolation purity. Sample processing is user friendly, requires no pre-preparation of the sample and enrichment of cells can be achieved with a single processing step. The microfluidic device is also label free and will allow for the retrieval of viable CTCs after blood processing. All together, the system is attractive for applications in oncology research.

2. Methods and materials

2.1. Sample blood collection

Informed consent from healthy volunteers and cancer patients were taken before blood extraction. Blood samples from healthy volunteers served as controls and were also used in spiking experiments. Samples were stored in EDTA tubes (BD, Franklin Lakes, NJ, USA) prior to use and discarded after the experiment. Institutional Review Board approval and informed consent was obtained from patients with lung cancer accrued onto this study. 10 ml of blood was extracted each time into sodium EDTA tubes after discarding the first 0.5–1 ml of blood drawn.

2.2. Microdevice fabrication

The fabrication of the microdevice was done via soft lithography (McDonald et al., 2000) and the procedures are briefly described below. The design of the microdevice was first printed on a photo mask (Infinite Graphics Inc., Minneapolis, MN, USA), drawn using the software Cadence (Cadence Design Systems, Inc., San Jose, CA, USA). SU8-2025 (Microchem Corporation, Newton, MA, USA) was spin coated at 3200 rpm for 45 s on an 8 in. silicon substrate to achieve the thickness of 18–20 μm. Then it underwent an ultraviolet exposure of 120 mJ/cm^2 through the photo mask and followed by the photoresist development. A final hard bake was performed to ensure better adhesion of the photoresist to the substrate to form the final master mold. Polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) mixed according to manufacturer's recommendation was degassed and poured over the master mold. The mixture was then subjected to the curing conditions of 80 °C for 2 h in an oven. Fluidic ports were created using punches on the patterned PDMS after the removal from the master mold. The PDMS block together with a glass slide were then subjected to oxygen plasma treatment and bonded irreversibly. Tubings were finally inserted directly into the fluidic ports of the finished device to allow samples to be introduced.

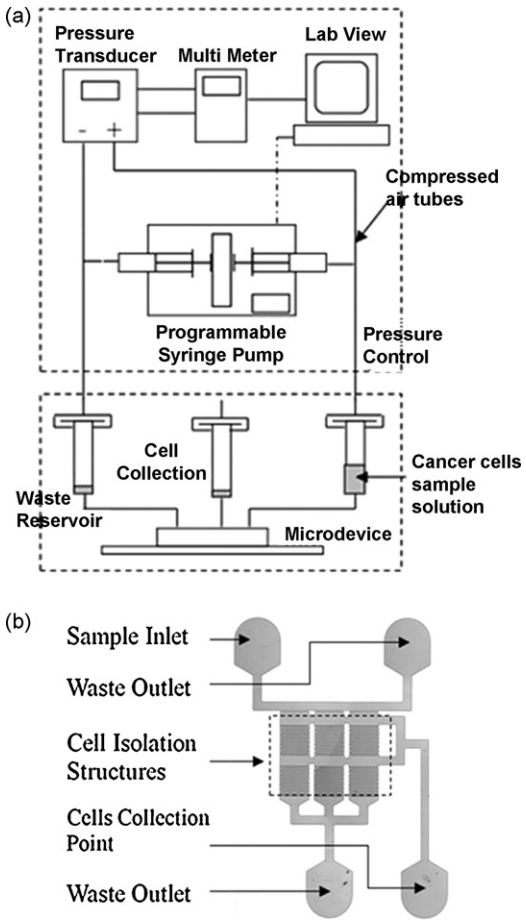

Fig. 1. Microdevice setup and design layout. (a) Schematic of semi-automated setup which is controlled by the computer to adjust the pressure differential to drive blood samples through the microdevice. (b) Overall microfluidic chip layout.

2.3. Experimental setup and apparatus preparation

The experimental setup and the design of the microdevice are shown in Fig. 1. The platform was upgraded from the previously reported (Tan et al., 2009) using manual pressure regulators. The current system used compressed air from two large syringes to produces pressured lines as shown in Fig. 1a. The differential pressure was measured by a pressure transducer which was feedback to the computer via a voltmeter to precisely control the pressure settings into the microdevice. A program written in NI Labview (NI, Austin, TX, USA) controlled the syringe pump (Harvard Apparatus, Holliston, MA, USA) and made minute adjustments every 100 ms in response to pressure drop. This allowed semi-automation in the blood processing and also made the entire system easily portable without requiring external pressure sources.

For apparatus preparation prior to samples processing, the microfluidic system was flushed with 5 mM EDTA (Sigma, St. Louis, MO, USA) buffer through the sample inlet as shown in Fig. 1b for 10 min at 120 μl/min. No other preparatory steps were required for the sample. The device was mounted on an inverted microscope (Leica Microsystems, Singapore) and cell enumeration was done manually after cell isolation. Image capturing of the cell isolation process was taken using a high speed camera (Photron, San Diego, CA, USA).