High-Throughput Immunomagnetic Cell Detection Using a Microaperture Chip System

Chun-Li Chang, Shadia I. Jalal, Wanfeng Huang, Aamer Mahmood, *Member, IEEE*, Daniela E. Matei, and Cagri A. Savran

***Abstract*— We report a microchip system based on a combination of immunomagnetic separation, microfluidics, and size-based filtration for high-throughput detection of rare cells. In this system, target cells bind to magnetic beads *in vitro* and flow parallel to a microchip with flow rates of milliliters/minute. A magnetic field draws the bead-bound cells toward the microchip, which contains apertures that allow passage of unbound beads while trapping the target cells. The cells captured on the chip can be investigated clearly under a microscope and released from the chip for further analysis. We first characterize the system by detecting cancer cell lines (MCF-7 and A549) in culture media. We then demonstrate detection of 100 MCF-7 cells spiked in 7.5 mL of human blood to simulate detection of circulating tumor cells present in cancer patient blood samples. On average, 85% of the spiked cells were detected. We expect this system to be highly useful in a wide variety of clinical as well as other applications that seek rare cells.**

***Index Terms*— CTC detection, immunomagnetic separation, microfluidics, rare cell detection, tumor cells, size-based filtration.**

I. Introduction

CELL sorting techniques are used to isolate whole cells from complex mixtures for detection, further enrichment and analysis [1]–[4]. The evolution of cell separation technologies has contributed to researchers' understanding of molecular biology of cells and benefited a wide range of fields including biology, medicine and pathology. Detecting cells at low concentrations is still challenging because finding them can require large amounts of samples. For example, detecting rare circulating tumor cells (CTCs) is difficult due to their low concentration, and hence a reliable analysis requires large volumes of blood (several mLs) [5]–[7]. The main goal of this study was to develop a robust and versatile system capable of detecting rare cells present in large fluid samples.

Currently one of the most widely used systems to detect whole cells is flow cytometry, which can separate and recover cells at high yield and purity. However, its volumetric throughput is generally low and the operational cost can be high which makes detecting rare cells a challenge [8].

In recent years, numerous microfluidic cell sorting systems were developed to detect rare cells that use fluorescence or magnetic assistance or both [9], [10]. For example, the Soh Group reported several systems for single- or multi-target cell sorting. In these systems the target cells were first labelled magnetically or dielectrophoretically with particles, and then were deflected in a flow by applying magnetic or electric field for separation [2]–[4]. The Toner and Haber Groups initially developed two systems for isolating CTCs by flowing blood samples through chips composed of micro-patterns. These micro-patterns were designed for optimal fluidic mixing and were functionalized with antibodies to capture target CTCs [5], [6]. They recently developed the microfluidic iChip system that integrates hydrodynamic cell sorting (for removal of red blood cells and platelets), inertial focusing, and magnetophoretic separation of white blood cells and CTCs [11]. Kang *et al.* reported a combined micromagnetic-microfluidic device for detection of CTCs from whole blood. In this system the sample flows through a long channel wherein the CTCs bound by magnetic-beads are drawn to a series of small wells and analyzed using fluorescent microscopy [12]. Takao and Takeda incorporated immunomagnetic separation and flow cytometry for not only detection of CTCs, but also discrimination between live and dead CTCs [13].

Another class of CTC detection systems is size-based filtration. Unlike most microfluidic or immunomagnetic systems that utilize affinity-based recognition, these systems rely on discriminating cells based on their size, by forcing the sample fluid through a porous membrane/filter. The filter has a precisely controlled pore size that allows the passage of smaller unwanted entities and retains the larger target cells [14]–[18].

We herein present a system which combines microfluidics, immunomagnetics and size-based separation (Fig. 1) for high-throughput rare cell detection [19]. In this system, the antibody-conjugated magnetic beads first bind with the target cells present in a sample fluid. The sample fluid then flows parallel to a micro-aperture chip while the bead-bound cells are attracted to the chip surface by means of a magnetic force. The bead-bound cells are larger than the apertures of the chip

Manuscript received January 7, 2014; revised April 22, 2014; accepted April 24, 2014. Date of publication April 30, 2014; date of current version July 22, 2014. This work was supported by the National Science Foundation under Grant 0925417, by the Purdue Oncological Sciences Center, by the Purdue Oncological Sciences Center, by the Purdue Center for Cancer Research, by Walther Cancer Foundation, and by the Indiana University Simon Cancer Center. The associate editor coordinating the review of this paper and approving it for publication was Prof. Sang-Seok Lee.

C.-L. Chang and W. Huang are with the Birck Nanotechnology Center, School of Mechanical Engineering, Purdue University, West Lafayette, IN 47907 USA (e-mail: chang64@purdue.edu; huang328@purdue.edu).

S. I. Jalal and D. E. Matei are with the Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202 USA (e-mail: sjalal@iu.edu; dmatei@iu.edu).

A. Mahmood is with the Birck Nanotechnology Center, Purdue University, West Lafayette, IN 47907 USA (e-mail: amahmood@purdue.edu).

C. A. Savran is with the Birck Nanotechnology Center, School of Mechanical Engineering, Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN 47907 USA (e-mail: savran@purdue.edu).

Color versions of one or more of the figures in this paper are available online at http://ieeexplore.ieee.org.

Digital Object Identifier 10.1109/JSEN.2014.2321167

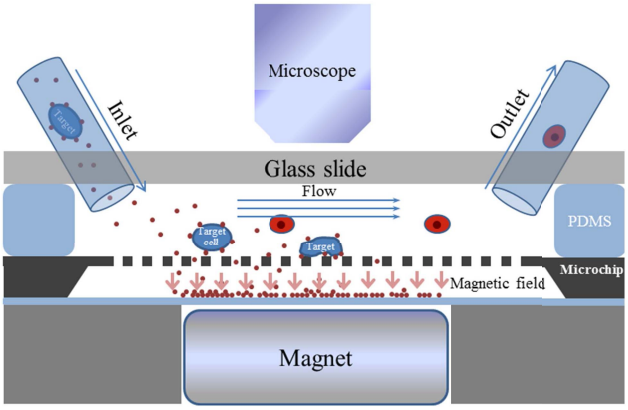

Fig. 1. Schematic of the micro-aperture chip system.

and hence stay on the chip surface while the smaller unbound beads which could interfere with the visualization process are filtered out. The system is robust, versatile, relatively easy to make and compatible with existing laboratory practices. Moreover, it can sustain a high volumetric throughput with flow rates as high as 4.3 mL/min, and can release the captured target cells for storage or further analysis which also allows re-using the chip.

II. Detection Strategy

A schematic diagram of the system is illustrated in Fig. 1. Magnetic beads are first conjugated with antibodies specific to the antigens over-expressed on the target cells, and are subsequently added into the sample fluid for incubation. The sample fluid is then introduced into a fluidic chamber which accommodates a micro-aperture chip composed of an array of through-holes that are larger than the beads but smaller than the target cells. While the sample fluid flows through the chamber and in parallel to the chip, a magnetic field is introduced to apply a vertical attractive force on the beads. The target cells bound with beads are pulled down and held on the chip surface by the magnetic field while other entities in the sample fluid are washed away. The free/unbound magnetic beads are pulled through the apertures by the magnetic field to the bottom section of the fluidic chamber. The microchip is then scanned while still in the chamber by a microscope to quantitate and/or analyze the cells. The captured cells can readily be retrieved for storage or further analysis by providing flow without applying magnetic field. Since the chip is not functionalized for specific cells, it can be re-used for subsequent experiments.

Although this system can be applied to detection of a wide variety of cells, in this study we used two cancer cell lines, MCF-7 (breast cancer) and A549 (lung cancer) as our model target cells to characterize our system. To recognize these two cell lines, antibodies against the epithelial cell adhesion molecule (anti-EpCAM) and the epidermal growth factor receptor (anti-EGFR) were used to functionalize the magnetic beads, respectively. For system characterization, the target cells were suspended in their culture media and incubated with antibody-beads for 1 hour at room temperature before flowing into the fluidic chamber. The size of the apertures on the chip

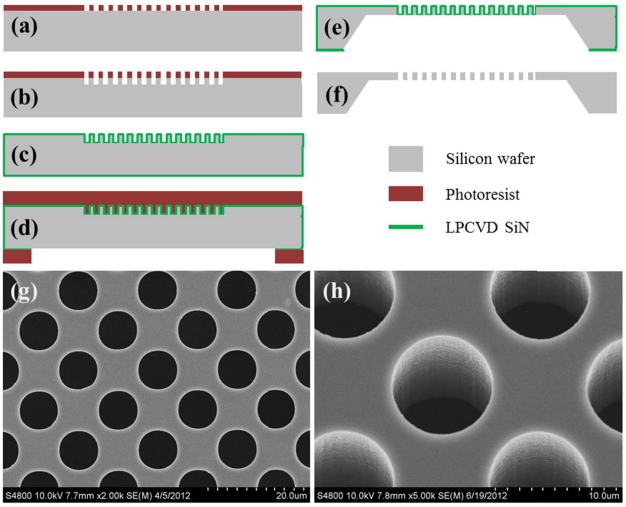

Fig. 2. (a)–(f) Fabrication process flow and (g–h) SEM images of a fabricated micro-aperture chip. Each aperture is 8 μm in diameter.

was 8 μm which is larger than the beads but smaller than both cancer cell lines whose diameters can vary between 10 and 30 μm. The fluidic chamber was subjected to bright-field microscopy to count the cells detected on the microchip.

To demonstrate the utility of the system in clinical applications, MCF-7 cells were spiked into blood samples obtained from healthy donors. Majority of the red blood cells (RBCs) were first removed using lysis buffer and the remaining sample was re-suspended in a phosphate buffered saline (PBS) solution and incubated with anti-EpCAM beads at 4 °C for over 1 hour. To increase the detection yield, the sample was circulated in the fluidic chamber at a flow rate of 2 mL/min for 4 minutes. The cells captured on the microchip were fluorescently labeled while in chamber with anti-cytokeratin-FITC (anti-CK-FITC to confirm cancer cells), anti-CD45-PE (to rule out leukocytes), and 4′,6-diamidino-2-phenylindole (DAPI to label nuclear DNA) and the chip surface was subjected to fluorescent microscopy.

III. Materials and Methods

A. Fabrication of Micro-Aperture Chips

The fabrication process flow of the micro-aperture chip is shown in Fig. 2(a)-(f). Photoresist (PR) AZ9260 (MicroChemicals GmbH) was first spin-coated onto the front side of a 500 μm-thick 4″ <100> silicon wafer followed by a soft-bake on a hot plate at 110 °C for 10 minutes. Subsequently the PR was exposed and developed using AZ400:DI water mixture (1:4 by volume). Deep Reactive Ion Etching (DRIE, STS ASE) using the Bosch process was used to selectively etch the silicon for 50 μm to create an array of cylindrical cavities. The PR was then removed using acetone and a layer of low stress silicon nitride (100 nm) was deposited on both sides of the wafer using low-pressure chemical vapor deposition (LPCVD). Once again AZ9260 was spin-coated, this time on the back side of the wafer, which was followed by a soft bake. The PR was patterned using back side alignment in a Karl Suss MA6 optical aligner to define a window for wet etching. The exposed silicon nitride was dry-etched using SF6 plasma (Plasma Tech RIE 80). Following that,

the wafer was placed in a 40% potassium hydroxide (KOH) solution at 80 °C for wet etching of the silicon from the back side. The process was carefully monitored and stopped when silicon was etched till the bottom of the holes (when light could transmit through the holes). The remaining nitride layer was stripped by immersing the wafer into HF:DI water = 1:10. Finally, the wafer was cleaned using a piranha solution (H_2SO_4:H_2O_2 = 3:7), ethanol and DI water to remove any residuals and particles. The area that contains arrays of micro-apertures is 9 mm × 3 mm in length and width, and is located at the center of a 40 mm × 20 mm chip. The SEM images of the micro-apertures on a fabricated chip are shown in Fig. 2 (g) and (h).

B. Cell Culture and Preparation of Cell Suspensions

The breast cancer cell line MCF-7, obtained from American Type Culture Collection (ATCC), was cultured in Eagle's Minimum Essential Medium (EMEM, ATCC) with 0.01 mg/ml bovine insulin (Sigma-Aldrich) and 10% fetal bovine serum (FBS, Gemini Bio Products); while the lung cancer cell line A549, obtained from Indiana University School of Medicine, was cultured in F-12K Medium (ATCC) with 10% FBS. Both cell lines were harvested using Trypsin-EDTA (Invitrogen) and re-suspended in culture media just before cell detection experiments. The original cell concentrations were measured using Countess Automated Cell Counter (Invitrogen) and/or hemocytometry. The cells were subsequently spiked into media or blood to achieve concentrations and volumes intended for detection experiments.

C. Blood Samples for MCF-7 Spiking

Blood donated by healthy volunteers were collected in BD vacutainer tubes with additives of either acid citrate dextrose (ACD) solution A or sodium polyanethol sulfonate (SPS) under an IRB-approved protocol. Blood samples were kept at 4 °C starting from immediately after collection until the spiking process. Blood samples were used within 12 hours after collection.

D. Anti-EpCAM and Anti-EGFR Beads

Antibodies against EpCAM and EGFR were conjugated to magnetic beads via biotin-streptavidin affinity, and were prepared freshly before detection. Biotinylated polyclonal antibodies against human EpCAM were purchased from R&D systems; while biotinylated monoclonal antibodies against human EGFR were purchased from Abcam. Streptavidin conjugated magnetic beads with 1 μm diameter were obtained from Sigma Aldrich. We saturated the magnetic beads (20 μL, 10 mg/mL) by incubating them with excess amount of antibodies (10 μL, 0.2 mg/mL) in PBS solution at room temperature for 1 hour, followed by rinsing with PBS three times on a magnetic stand and re-suspending in PBS.

E. Labeling of Captured Cells for Fluorescent Microscopy

Cells captured on the microchip surface were labeled fluorescently while the microchip was in the fluidic chamber. A 4% paraformaldehyde (PFA) solution in PBS was first introduced into the chamber and left for 20 minutes at room temperature. Anti-pan Cytokeratin monoclonal antibodies conjugated with FITC (Abcam, USA), anti-CD45 monoclonal antibodies conjugated with phycoerythrin (Abcam, USA), and DAPI (Sigma-Aldrich, USA) were then introduced into the chamber and incubated for 1 hour at room temperature to label the cells.

F. Experimental Setup

A fluidic chamber (Fig. 1) was first made by placing a layer of Polydimethylsiloxane (PDMS, ∼1.5 mm thick) on a microchip as a spacer and covered with a 1 mm thick glass slide. The dimension of the fluidic channel that encloses the porous area was defined by patterning the PDMS to have a 30 mm by 3.8 mm grove. The bottom of the microchip was also sealed by attaching a thin layer of PDMS (∼0.1 mm thick).

The assembled fluidic chamber was mounted on a hollow acrylic stand, in which a neodymium permanent magnet (K&J Magnetics, grade N52 with a flux density of 6,451 Gauss at the magnet surface) was inserted to provide a magnetic field. By using a peristaltic pump (Ismatec ISM596B), sample fluids were flowed through the fluidic chamber via an inlet and outlet opened on the cover glass. The inlet of the chamber was connected to the fluidic sources while the outlet was connected to the pump and drained into a waste container or a collection tube.

IV. Results and Discussion

A. Characterization of the System

1) Detection Yield vs Incubation Time: The incubation time needed for beads to interact with the target cells depends on various parameters such as the number of beads, the sample volume, incubation temperature, number of antibodies on beads and their affinity for the antigens. We found that for the specific case of 50 MCF-7 cells in 1 mL culture medium with 20 μg of antibody-beads, processed with a flow rate of 3 mL/min, an incubation time of 35-40 minutes can be sufficient for a yield of 90% or higher and that longer incubation times do not increase the yield significantly. When we increased the sample volume to 10 mL while keeping other parameters the same, the yield was as low as 30% for a 40 minute incubation time (while 60 minutes resulted in over 90% yield). Increasing the bead amount by 4 fold (despite a 10 fold increase in sample volume) increased the yield back to about 83% for 40-minute incubation confirming that incubation times can be effectively shortened by increasing the amount of beads used.

2) Detection Yield vs Flow Rate: We next studied the effect of flow rate on capture yield. 2 μL (20 μg) of antibody-coupled beads were added into 1 mL of cell suspensions (concentration: 50 cells/mL) with the following antibody/cell line combinations: (1) anti-EpCAM/MCF-7, (2) anti-EpCAM/A549, and (3) anti-EGFR/A549. The suspensions were incubated with the beads at room temperature for 1 hour. The samples were then run through the fluidic chamber at flow

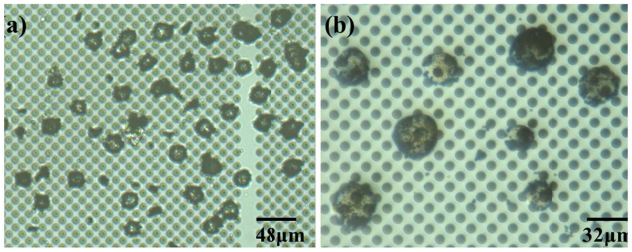

Fig. 3. Optical micrographs of the chip surface with (a) MCF-7 cells bound by anti-EpCAM beads and (b) A549 cells bound by anti-EGFR beads.

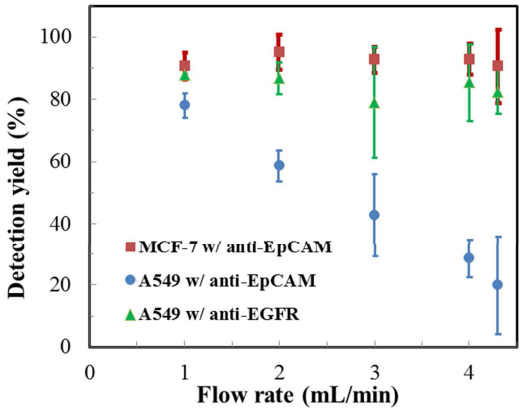

Fig. 4. Detection yield of MCF-7 and A549 cells using anti-EpCAM and anti-EGFR beads with respect to flow rate. Error bars indicate standard deviations for three measurements.

rates ranging from 1 to 4.3 mL/min while a magnetic field was applied below the chamber. The chip was immediately washed with 1 mL of PBS at the same flow rate. The microchip was then inspected while still in the chamber using bright-field microscopy to enumerate the detected cells. Fig. 3 (a) and (b) respectively show the micrographs of the chip that contains MCF-7 cells bound with anti-EpCAM beads, and A549 cells bound with anti-EGFR beads that were trapped by the magnetic field when the samples were flowing at a rate of 3 mL/min. There were minimal amount of impurities on the chip (free beads or cell debris) and the trapped cells were easily distinguishable. The detection yields were calculated by normalizing the number of cells detected by that added into the media, and plotted against flow rates in fig. 4. When we study the detection yields obtained by using anti-EpCAM beads, we notice that the yield for A549 decreased from 78% to 20% when the flow rate increased from 1 to 4.3 mL/min (max flow rate of our pump), whereas the yield of MCF-7 remained above 90% regardless of the flow rate. This indicates that more anti-EpCAM beads were bound to the MCF-7 cells than the A549 cells. As a result, the magnetic force could draw the MCF-7 cells to the chip surface at high flow rates which washed away some of the A549 cells. However, when anti-EGFR beads are used, the A549 cells could be detected with 80% to 90% yield regardless of the flow rate indicating that more anti-EGFR beads were bound to A549 cells than the anti-EpCAM beads under similar conditions. This could potentially be attributed to either higher EGFR over-expression (than EpCAM) on the particular A549 cells, or higher affinity of the anti-EGFR antibodies for the cells.

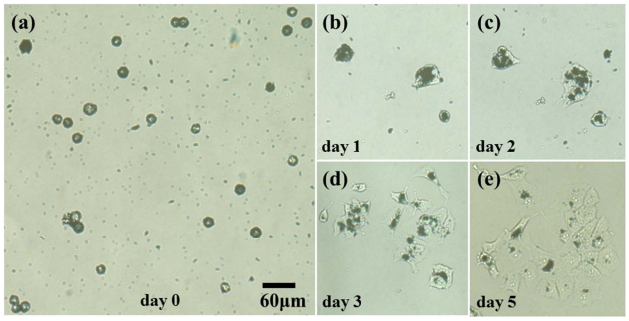

Fig. 5. Post-detection retrieval and growth of MCF-7 cells. (a) Cells retrieved from the microchip and placed in a Petri dish. (b)–(e) Cell growth at the same location monitored from day 1 to day 5.

B. Retrieval and Cultivation of the Detected MCF-7 Cells

To demonstrate the ability of our system to readily release the detected cells from the chip surface for further usage and analysis, we again used MCF-7 as our model targets. 100 MCF-7 cells suspended in 1 mL of medium were first detected using the microchip system at a flow rate of 3 mL/min, following the same protocol as before. Next, the magnet was removed from underneath the chip and 1 mL PBS solution was flowed through the chamber at 3 mL/min to wash the trapped cells away from the chip surface. The fluid was collected at the outlet of the chamber, in a 35-mm Petri dish. The image of the retrieved cells in the Petri dish is shown in Fig. 5 (a). The collected cells can be stored for further analyses such as cell enrichment or DNA/RNA detection. In this study we investigated culturing of the retrieved cells. The growth of the cells as monitored over a few days is shown in Fig. 5 (b)-(e). The bead-bound cells first settled down and attached to the dish on day 1. From day 2 onwards, the cells started to grow and divide. The morphology of the cells looked similar to that of the parental cell line prior to the experiment. The results suggest that the cells bound with anti-EpCAM beads are still able to grow in an appropriate environment.

C. Detection in Large Sample Volumes

We next investigated the ability of our system to detect rare cells present in large volumes of samples. We began with 50 MCF-7 cells in 7.5 mL, and then reduced the cell number and increased the sample volume to obtain 30 cells in 10 mL and 10 cells in 12 mL, respectively. The corresponding concentrations were 6.7, 3.0 and 0.8 cells/mL. With a flow rate of 3 mL/min, the resulting detection yields were 92.7%, 92.3% and 90.0%, respectively, which are in agreement with our characterization data. The experimental results, summarized in Table 1, demonstrate the ability of the system to perform high-throughput analysis of cells present in large sample volumes. It took only 4 minutes to run 12 mL of sample through the fluidic chamber, and the overall experiment, including one hour incubation, was less than 70 minutes. As described earlier, incubation time can be shortened by using more beads.

D. Detection of MCF-7 Cells Spiked in Blood

To investigate the clinical applicability of this system to the medical field, we captured tumor cells in blood in a manner

TABLE I
LARGE VOLUME DETECTION

# of Cells Added	Volume (mL)	Concentration (cells/mL)	Average # of Cells Detected*	Average Detection Yield (%)*	STDEV (%)*
50	7.5	6.7	46.3	92.7	11
30	10	3.0	27.7	92.3	6.9
10	12	0.8	9.0	90.0	10

* Data obtained from 3 measurements

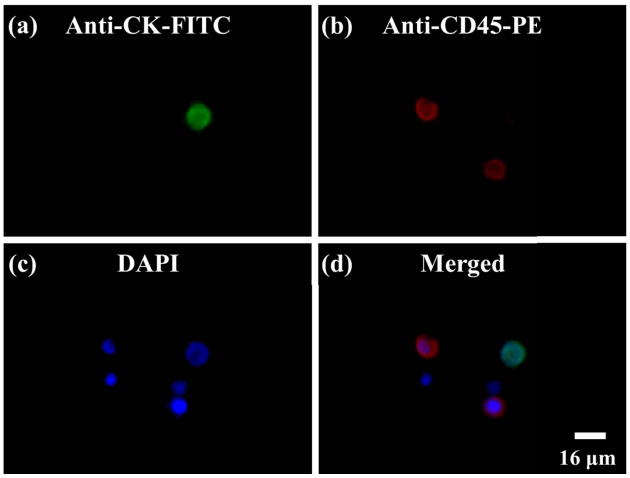

Fig. 6. Fluorescent micrographs of cells detected from MCF-7 spiked blood on a microchip: (a) a MCF-7 cell stained by CK-FITC, (b) leukocytes stained by CD45-PE, (c) cell nuclei stained by DAPI, and (d) a merged image of (a)-(c).

that mimics capturing rare CTCs in blood samples of cancer patients. We spiked 100 MCF-7 cells in 7.5 mL of blood collected from healthy donors under an approved IRB protocol and detected the cells using our system. A spiked blood sample was first treated with RBC lysis buffer to remove majority of the RBCs. The remaining cells were re-suspended in 1 mL of PBS buffer and incubated with 3μL (30 μg) of anti-EpCAM beads at 4 °C for about 1 hour. The sample was then circulated in the fluidic chamber with the presence of a magnetic field at a flow rate of 2 mL/min for 4 minutes, following by washing with 2 mL of PBS solution. A fixation solution (4% PFA in PBS) was subsequently introduced into the chamber to fix the cells captured on the chip. The fixed cells were stained fluorescently with anti-CK-FITC, anti-CD45-PE, and DAPI as described before. The microchip was then inspected while still in the chamber using a fluorescent microscope to identify and enumerate the MCF-7 cells. Fig. 6 shows the fluorescent images of an area on a microchip that contains a few cells. The anti-CK-FITC bound to the MCF-7 cells emitted green fluorescent light (fig. 6 (a)), while the anti-CD45-PE bound to the leukocytes emitted red light (fig. 6 (b)). DAPI stained the cell nuclei and emitted blue light (fig. 6 (c)). We identified MCF-7 cells based on a combination of factors including the size (10-30 μm) and shape (close to circular) of the cells and their fluorescent emissions (CK+, DAPI+ and CD45-). The average detection yield from 3 measurements was 85.4% with a standard deviation of 1.5%, which is in good agreement with the results of both the characterization experiments and large volume detection experiments conducted in culture media. Using the above protocol, the total time needed to process a 7.5 mL of blood sample for rare cell capture, including lysing RBCs (∼20 min), incubating with antibody-beads (60 min), and flowing/circulating in the fluidic chamber (<5min), was 85 min. On-chip fluorescent detection requires an additional 80 minutes for fixation and staining. The results demonstrate that this system could be readily applied to high-throughput detection of CTCs in clinically-relevant amounts of patient blood samples.

V. CONCLUSION

We have demonstrated a micro-aperture chip system for high-throughput detection of rare cells. The system is based on a combination of immunomagnetic separation, micro-fluidics and size-based filtration, and is capable of detecting rare cells present in standard (7.5 mL) and larger sample volumes. Moreover, the detected cells can readily be retrieved for further usage, and the chip can be re-used for subsequent experiments. We showed that the MCF-7 cells collected from the microchip can later be cultivated in a Petri dish. With this system, detection of 10 MCF-7 cells in 12 mL of culture media (0.8 cell/mL) was possible with 90% yield. We also showed that 85% of the 100 MCF-7 cells spiked in 7.5 mL of blood can be detected using this system, which demonstrates the potential of this system in detecting CTCs. Using the current protocol, a typical 7.5 mL of blood can be processed in less than 90 min to capture rare cells. The cells captured on the microchip can subsequently be analyzed on-chip or retrieved for further analysis. The concept of this system is generic and can be modified to detect a wide variety of cells present in other bodily fluids. We envision that this versatile and robust system can have a wide range of medical, as well as environmental applications.

REFERENCES

[1] M. Zborowski and J. J. Chalmers, "Rare cell separation and analysis by magnetic sorting," *Anal. Chem.*, vol. 83, no. 21, pp. 8050–8056, Nov. 2011.
[2] J. D. Adams, U. Kim, and H. T. Soh, "Multitarget magnetic activated cell sorter," *Proc. Nat. Acad. Sci. USA*, vol. 105, no. 47, pp. 18165–18170, Nov. 2008.
[3] X. Hu, P. H. Bessette, J. Qian, C. D. Meinhart, P. S. Daugherty, and H. T. Soh, "Marker-specific sorting of rare cells using dielectrophoresis," *Proc. Nat. Acad. Sci. USA*, vol. 102, no. 44, pp. 15757–15761, Nov. 2005.
[4] U. Kim and H. T. Soh, "Simultaneous sorting of multiple bacterial targets using integrated dielectrophoretic-magnetic activated cell sorter," *Lab Chip*, vol. 9, no. 16, pp. 2313–2318, Aug. 2009.
[5] S. Nagrath *et al.*, "Isolation of rare circulating tumour cells in cancer patients by microchip technology," *Nature*, vol. 450, no. 7173, pp. 1235–1239, Dec. 2007.
[6] S. L. Stott *et al.*, "Isolation of circulating tumor cells using a microvortex-generating herringbone-chip," *Proc. Nat. Acad. Sci. USA*, vol. 107, no. 43, pp. 18392–18397, Oct. 2010.
[7] F. A. Coumans, S. T. Ligthart, J. W. Uhr, and L. W. Terstappen, "Challenges in the enumeration and phenotyping of CTC," *Clinical Cancer Res.*, vol. 18, no. 20, pp. 5711–5718, Oct. 2012.
[8] S. H. Cho, J. M. Godin, C.-H. Chen, W. Qiao, H. Lee, and Y.-H. Lo, "Review article: Recent advancements in optofluidic flow cytometer," *Biomicrofluidics*, vol. 4, no. 4, p. 043001, Dec. 2010.

[9] J. Chen, J. Li, and Y. Sun, "Microfluidic approaches for cancer cell detection, characterization, and separation," *Lab Chip*, vol. 12, no. 10, pp. 1753–1767, Apr. 2012.
[10] P. Li, Z. S. Stratton, M. Dao, J. Ritz, and T. J. Huang, "Probing circulating tumor cells in microfluidics," *Lab Chip*, vol. 13, no. 4, pp. 602–609, Feb. 2013.
[11] E. Ozkumur *et al.*, "Inertial focusing for tumor antigen-dependent and-independent sorting of rare circulating tumor cells," *Sci. Transl. Med.*, vol. 5, no. 179, p. 179ra47, Apr. 2013.
[12] J. H. Kang, S. Krause, H. Tobin, A. Mammoto, M. Kanapathipillai, and D. E. Ingber, "A combined micromagnetic-microfluidic device for rapid capture and culture of rare circulating tumor cells," *Lab Chip*, vol. 12, no. 12, pp. 2175–2181, Jun. 2012.
[13] M. Takao and K. Takeda, "Enumeration, characterization, and collection of intact circulating tumor cells by cross contamination-free flow cytometry," *Cytometry A*, vol. 79, no. 2, pp. 107–117, Feb. 2011.
[14] M. Hosokawa *et al.*, "Size-selective microcavity array for rapid and efficient detection of circulating tumor cells," *Anal. Chem.*, vol. 82, no. 15, pp. 6629–6635, Aug. 2010.
[15] S. Zheng *et al.*, "Membrane microfilter device for selective capture, electrolysis and genomic analysis of human circulating tumor cells," *J. Chromatography A*, vol. 1162, no. 2, pp. 154–161, Aug. 2007.
[16] S. Zheng *et al.*, "3D microfilter device for viable circulating tumor cell (CTC) enrichment from blood," *Biomed. Microdevices*, vol. 13, no. 1, pp. 203–213, Feb. 2011.
[17] M. Alunni-Fabbroni and M. T. Sandri, "Circulating tumour cells in clinical practice: Methods of detection and possible characterization," *Methods*, vol. 50, no. 4, pp. 289–297, Apr. 2010.
[18] L. S. Lim *et al.*, "Microsieve lab-chip device for rapid enumeration and fluorescence *in situ* hybridization of circulating tumor cells," *Lab Chip*, vol. 12, no. 21, pp. 4388–4396, Nov. 2012.
[19] C. L. Chang, C. A. Savran, S. Jalal, and D. E. Matei, "Micro-aperture chip system for high-throughput immunomagnetic cell detection," in *Proc. IEEE Sensors*, Oct. 2012, pp. 1672–1675.

Chun-Li Chang received the B.S. degree in electrophysics and the M.S. degree in electronics engineering from National Chiao Tung University, Hsinchu, Taiwan, in 2001 and 2003, respectively, and the Ph.D. degree in electrical and computer engineering from Purdue University, West Lafayette, IN, USA, in 2009.

He is currently a Visiting Assistant Professor with the School of Mechanical Engineering, Purdue University. His research interests include the development of biosensors and their applications to cellular and biomolecular detection.

Shadia I. Jalal received the M.B.B.S. degree from the University of Jordan, Amman, Jordan, in 2002. She completed residency training in internal medicine and fellowship training in hematology oncology at Indiana University, Bloomington, IN, USA, in 2006 and 2009, respectively. She is currently an Assistant Professor of Medicine with the Division of Hematology Oncology, Indiana University School of Medicine, Indianapolis, IN, USA. She is a Physician Scientist involved in the research on thoracic malignancies, in particular, lung and esophageal cancers.

Wanfeng Huang received the B.S. degree in measurement and control technology and instrumentation from Beijing Jiaotong University, Beijing, China, in 2007, and the M.S. degree in instrumental science and technology from Tsinghua University, Beijing, in 2010.

He is currently pursuing the Ph.D. degree at the School of Mechanical Engineering, Purdue University, West Lafayette, IN, USA. His research focuses on application of biosensors for cell and biomolecular detection, and the simulation of multiphysics environment.

Aamer Mahmood (M'07) received the B.E. (with Hons.) degree in electrical engineering from the North West Frontier Province University of Engineering and Technology, Peshawar, Pakistan, in 1992; the M.S. degree in electrical engineering from the University of Engineering and Technology, Lahore, Pakistan, in 1999; and the Ph.D. degree in electrical engineering from the University of Texas at Arlington, USA, in 2006. After graduation, he continued post-doctoral research with the School of Electrical and Computer Engineering, Purdue University, West Lafayette, IN, USA. He joined the Birck Nanotechnology Center, Purdue University, as a Research Engineer, in 2008. He was promoted to Research Scientist and Senior Process Scientist in 2011 and 2012, respectively. His current research is focused on the development of novel microsensors and actuators. He has authored over 20 peer-reviewed articles in journals and conference proceedings.

Daniela E. Matei is an Associate Professor with the Indiana University Medical School, Indianapolis, IN, USA, and a member of the Divisions of Hematology-Oncology and Gynecologic-Oncology. She received the M.D. degree from the University of Medicine and Pharmacy, Bucharest, Romania. She completed an Internal Medicine residency at the State University of New York at Stony Brook, Stony Brook, NY, USA, and an Hematology-Oncology Research Fellowship at the University of California at Los Angeles, Los Angeles, CA, USA, where she trained at the Dr. David Chang's Laboratory, involved in gene expression profiling in ovarian cancer.

Dr. Matei is the Co-Leader of the Experimental and Developmental Therapeutics Group of the Indiana University Simon Cancer Center, Indianapolis. She was a recipient of the Research Scholar Award from the American Cancer Society, the VA Merit Award from the U.S. Department of Veteran Affairs, awards from the National Cancer Institute, and other peer-reviewed research grants. Her laboratory studies the role of tissue transglutaminase in cancer and other mechanisms of ovarian cancer metastasis. She is also involved in translational cancer research, and serves as the principal investigator and/or co-investigator on many clinical trials for ovarian cancer, including several National Cancer Institute-sponsored trials for gynecologic cancer. An important direction of investigation is epigenetic modulation as a method of resensitization to platinum in ovarian cancer.

Cagri A. Savran received the B.S. degree from Purdue University, West Lafayette, IN, USA, in 1998, and the S.M. and Ph.D. degrees in mechanical engineering from the Massachusetts Institute of Technology, Cambridge, MA, USA, in 2004. He is currently an Associate Professor of Mechanical Engineering with Purdue University, with a courtesy appointment at the Weldon School of Biomedical Engineering. His research focuses on applications of MEMS and nanotechnology to biological systems with a specific interest in cancer detection.