Development of microfluidic device and system for breast cancer cell fluorescence detection

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A biomicrofluidic device and a compact cellular testing system were developed to be used in cancer diagnostics. The device was fabricated by lithography-based microfabrication techniques, followed by two-step etching of deep reactive ion etching, and channels were formed by anodic bonding of Si and Pyrex. The device is based on the capture of cells inside a new meandering weir-type filter design, followed by detection and characterization using specific fluorescent labeling. Breast cancer cells MCF-7 and control cells MCF-10A were flowed through the microfluidic channels, and captured by meandering weir-type filters. 17β-Estradiaol(E_2)-BSA (bovine serum albumin)-FITC (fluorescein isothiocyanate) macromolecular complex was found to selectively label MCF-7, potentially serving as a cancer cell detection marker. MCF-7 cells were detected with specific and strong FITC signals after only 4 min of contact with the stain. The signals were about seven times stronger than that of a labeling performed on conventional glass slides. These results strongly suggest that this novel design has a potential application to detect cancer cells or other diseased cells without compromising the advantage of high sensitivity of the microfluidic approach. © *2009 American Vacuum Society.* [DOI: 10.1116/1.3049529]

I. INTRODUCTION

Breast cancer is one of the most common diseases in women,[1] about one in every eight women. Nowadays, clinical diagnostic approaches of breast cancer include palpation, mammography, ultrasonography, x-ray, and needle biopsy.[2] However these techniques do not sense abnormal cell growth until they become cancerous and invade nearby tissues. A simple quantitative analysis of cancer cells detected inside blood streams or body fluids can significantly help the clinician to generate a diagnosis and plan treatment as early as possible. We propose a microfluidics lab-on-chip (LOC) device for cancer cell detection for potential application in early cancer diagnostics.

Microfluidics approaches or LOC devices have been demonstrated as a suitable platform to study biomolecules, such as nucleic acids,[3] proteins, or cells.[4] More particularly, cell detection can be performed efficiently with microfluidics, in systems that can first capture the cells of interest and then identify them specifically as the targeted cells. Generally, current approaches require either additional chemical treatment on the surface of the microfluidic devices[6] or beads,[7] or additional complicated experimental setup.[8] We developed a fast, straightforward, and sensitive approach for breast cancer cell detection. Both cancer cells and control cells were mixed and pumped into the LOC. They were captured by micromechanical weir-type filters, and detected by a fluorescent marker that selectively binds to receptors of the cancer cells. The liguand estradiol (E_2), is an oestrogen, one of the predominant circulating ovarian steroid and the most biologically active hormone in breast tissue.[5] E_2 binds to specific oestrogen receptors (ER). It is known that MCF-7 cells express those receptors whereas MCF-10A cells are negative.[6] They are found in the nucleus and the cytosol, and the complex E_2-BSA-FITC has been used to identify more specifically those localized in the membrane.[7]

II. EXPERIMENTAL STUDY

A. Cell lines, buffers, and dyes

Two different cells, MCF-7 (breast cancer cells) and MCF-10A (used as control noncancerous breast cells) were obtained from American Type Culture Collection (ATCC, Manassas, VA 20108 USA) and cultured following the supplier's protocols. MCF-10A is a nontumorigenic epithelial cell line derived from the benign breast tissue of a 36-year-old Caucasian woman with fibrocystic disease. These cells are immortal, but otherwise normal, noncancerous mammary epithelial cells. MCF-7 is a corresponding human breast cancer cell line (adenocarcinoma), obtained from pleural effusion of a 69-year-old Caucasian female. These

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TABLE I. Comparison between MCF-7 and MCF-10A and their response against labels.

Cell No.	Cell-type	Reaction with E_2-BSA-FITC	Dye color	Diameter (μm)
MCF-7	Cancer cell	Yes	Green	15–20
MCF-10A	Normal cell	No	Blue	15–20

cells are nonmotile, nonmetastasic epithelia cancer cells. The diameters of MCF-7 and MCF-10A are about the same, 15–20 μm. Phosphate-buffer saline (PBS) buffer was filtered through 0.45 μm membrane filter (Millipore, Cork, Ireland) prior to use. Hoescht 33342 (labeling the cell nucleus in blue) was obtained from Invitrogen, Inc. (Carlsbad, CA). 17β-estradiol-bovine serum albumin-fluorescein isothiocyanate macromolecular complex (E_2-BSA-FITC) was obtained from Sigma-Aldrich (St. Louis, MO). This fluorescent complex can bind to oestrogen receptors on the cell membrane of MCF-7 cancer cells,[7,8] whereas MCF-10A cells are negative for these receptors.[6] Table I shows the comparison of the biological and physical properties between MCF-7 cancer cells and MCF-10A nontumorigenic control cells.

B. Design of microfluidic devices

Figure 1 shows the top view of the microfluidic device, while Fig. 2 shows the cross section of the microfluidic device. Ellipse microfluidic chamber is used to avoid air bubbles when the fluid flow through, as air bubble can be easily produced in the corner of rectangular chamber. It comprises one inlet, one outlet, and three chambers separated by two set of filters with a channel depth of 50 μm. The first set of filters is made of pillar-type structures, and is used to stop unwanted big particles from going into the meandering trapping chamber (second chamber). The width and length of the rain-drop pillars are 45 and 90 μm, respectively, while the spacing between the pillars is 30 μm. The second set of filters is made of meandering weir-type structures with a gap of 8 μm between the weirs and the glass cover, which serves as the capturing area. They are used to capture cells of diameter of 10–20 μm, which is larger than the gap. In order to optimize the cell capture, the meandering-type structures are

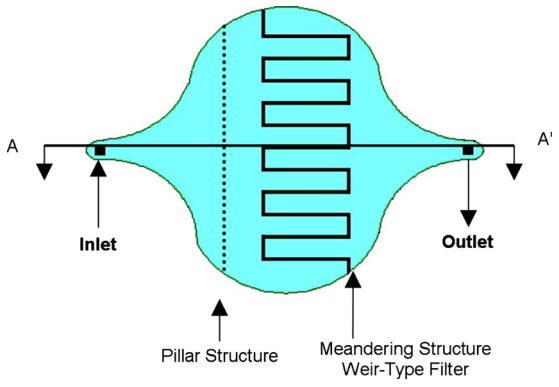

FIG. 1. (Color online) Schematic of top view of the microfluidics device.

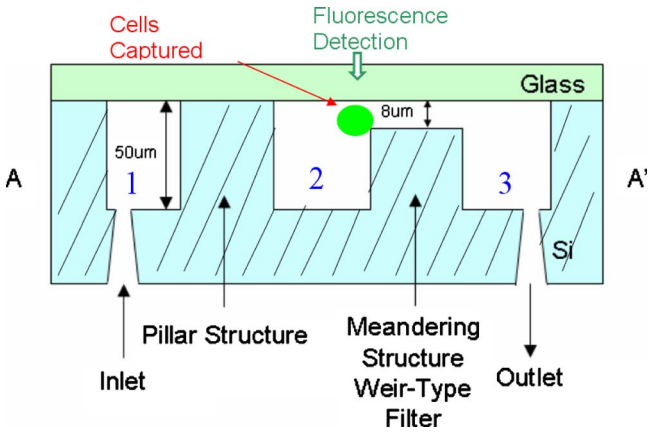

FIG. 2. (Color online) Schematic of cross section of microfluidics device, and chamber 2 is used for capturing microparticles.

used to increase the area for trapping compared to one straight-line weir-type filter. The capturing area can be increased by a factor of 2 or 3, depending on the length and the width of the meandering weirs. At a constant flow rate, it is also useful to reduce the fluidic resistance so as to avoid leakage by maintaining low pressures. This is because (1) fluidic resistance is negative related to the area of fluid path (area is total length of the weir multiplied by gap), and therefore negative related to the total length of the weir, while the gap is fixed at 8 μm; (2) the total length of the meandering-type weirs is about two to three times of that of the straight-line weirs.

C. Fabrication of microfluidic devices

The microfluidic devices were fabricated by 8 in. wafer batch process. It consists of a 750-μm-thick Si substrate and a 500-μm-thick Pyrex glass top cover. The microfluidic channels were produced by two-step deep reactive ion etching (DRIE) by STS (STS PLC., Newport, UK) using two masks in UV lithography on Si wafers, which were finally anodically bonded with Pyrex covers. The first mask defined the patterns of the channels using an oxide hard mask. Silicon was etched by SF_6 in STS DRIE machine after patterning. Figure 3(c) shows the scanning electron microscope (SEM) images of the first set of Si pillars according to the first mask. The second mask defined the meandering structure weir-type patterns, the height of which is 8 μm lower than the first set of pillars. Similar silicon etching by SF_6 using DRIE approach was followed. Figure 3(d) shows the SEM images of part of the second set of meandering silicon pillars according to the second mask. The inlet and outlet were silicon V-groove from the back side of the silicon wafer, which is opened by KOH wet etching from the back side of the Si wafer with double-side alignment mask patterning. Finally the patterned and etched Si wafer was bonded with a Pyrex glass wafer as top cover using anodic bonding. This fabrication process was adapted from a DNA purification chip.[9] The 8 in. Si-Pyrex bonded wafers (images not shown) were diced into LOC of 12×18 mm^2 for testing. Figure 3(b) shows the microfluidics LOC device in hand.

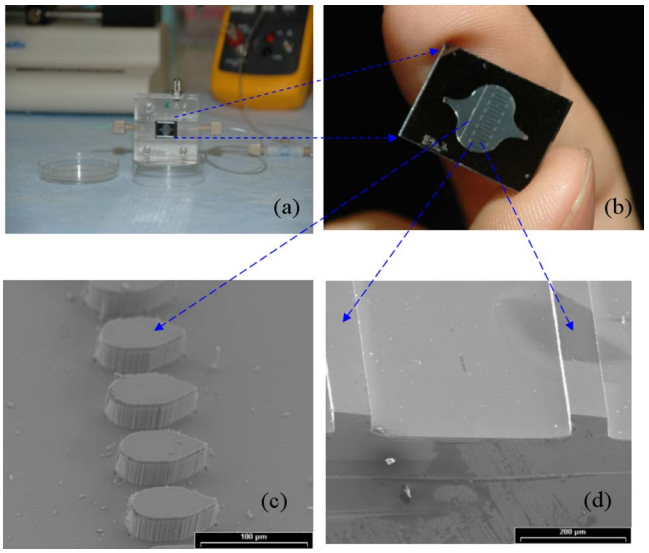

FIG. 3. (Color online) Image of a microfluidic device (a) inside the plastic testing holder connected to the syringe pump and the pressure gauge and (b) the device in hand; SEM images of (c) the silicon pillars inside the microfluidics device, and (d) part of meandering U-shaped silicon weirs (for cell capture) while the Pyrex cover is opened.

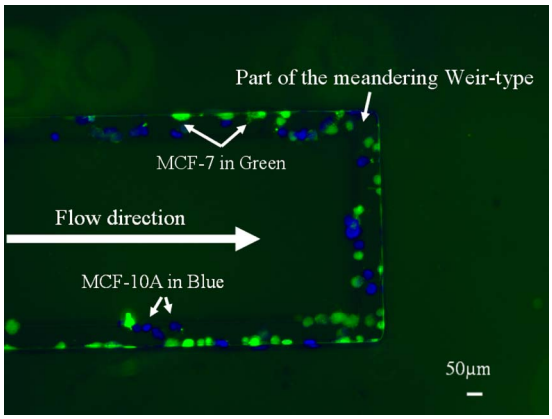

FIG. 4. (Color online) Overlap of two fluorescence images of the top view of the U-shape meandering weirs. One image was taken with the filter set for the blue Hoechst dye for MCF-10A, while the other one was taken with the filter set for the green FITC dye for MCF-7. Both captured cancer cells MCF-7 and control cells MCF-10A are shown as the arrow indicated.

III. TESTING, RESULTS, AND DISCUSSIONS

A customized plastic holder was designed to mount the device as shown in Fig. 3(a). It enables the injection of the washing buffers, bimolecular fluidic samples, and dyeing solutions in microlitre volumes by an automatic syringe pump (KDS100, KD Scientific, Boston, MA). The microfludic device is clamped mechanically by screws, between the top cover and the plastic chamber. Rubble O-rings (images not shown) were also used between the silicon device and the plastic chamber, to make sure no leakage. First, PBS was used to flush the device at a flow rate of 50 μl/min for 2 min prior to biosample injection into the device. PBS buffer was prepared and filtered through a 0.45 μm membrane filter (Millipore, Cork, Ireland) prior to use.

MCF-10A control cells were dyed following standard labeling procedures with 1 μl of nucleus dye Hoechst 33342. The nucleus of MCF-10A control cells were dyed with Hoechst 33342 and show in blue (emission peak at ~420 nm) in the fluorescent images, while that of MCF-7 cancer cells were not. 500 μl of a mixture of approximately 2000 dyed MCF-10A cells and approximately 2000 MCF-7 cells were injected into the device at a flow rate of 50 μl/min. Under the fluorescence microscope, the cells with fluorescent markers, was moving from the inlet toward first filters, and thereafter trapped at the second weir-type filters.

Immediately after that, the label 0.1 μM E_2-BSA-FITC (green, emission peak at about 520 nm) in PBS was introduced at a flow rate of 20 μl/min to selectively label the target cancer cell MCF-7. E_2-BSA-FITC was flushed through the device, reacting with the cells for 1–7 min in the second chamber at room temperature. E_2-BSA-FITC only binds with the ER side on the membrane of MCF-7 cancer cells, and therefore stain MCF-7, but not MCF10A. Therefore those cells trapped with FITC green fluorescence signals are MCF-7 cancer cells, while MCF-10A shows blue fluorescence signal from Hoechst 33342. During the experiments, the captured cells were monitored by fluorescence imaging. Once the pumping was finished, the final fluorescence images were taken using an upright Olympus BX61 fluorescence microscope with an exposure of 100 ms and analyzed using computer software IMAGE-PRO 6.1 (Media Cybernetics, Bethesda, MD). The gray level images were artificially colored with the software to match the respective colors of filters used.

Figure 4 is an overlap between two fluorescent images (using two respective filters, blue and green) of the top view of the U-shape meandering weirs, showing the blue and green labels. It confirms that E_2-BSA-FITC can be used as a specific marker for MCF-7 cancer cells, and does not label MCF-10A control noncancerous cells, in agreement with previous data.[6] In a final step, PBS was flushed through the device at a flow rate of 50 μl/min for 10 min as a washing step. This indicates the cell capture capability of the LOC: both cells can be captured at the same time. It also shows that this microfluidic device can be used to capture deformable breast cells that are about twice as big as the gap.

The labeling kinetics of E_2-BSA-FITC on MCF-7 has been studied on both Si/Glass microfluidic device and conventional glass slides. The fluorescence intensity was measured every minute for 7 min, using the same exposure time of 100 ms. It is found that the fluorescence signal (from 0 to 4096 for a 12 bit fluorescence image) increased from 1500 to 3500 in the first 4 min, and entered into a plateau at 3500–3700. This is due to the saturation of the binding sites on the cancer cell membrane by the dye E_2-BSA-FITC. A parallel test was conducted on a conventional glass slide, onto which 10 μl of a suspension of cancer cells at the same concentration was deposited, and incubated with a solution of 0.1 μM E_2-BSA-FITC for up to 7 min. A similar fluorescence signal was determined after every minute during the incubation. Figure 5 shows at the fourth minute, the fluorescent signal

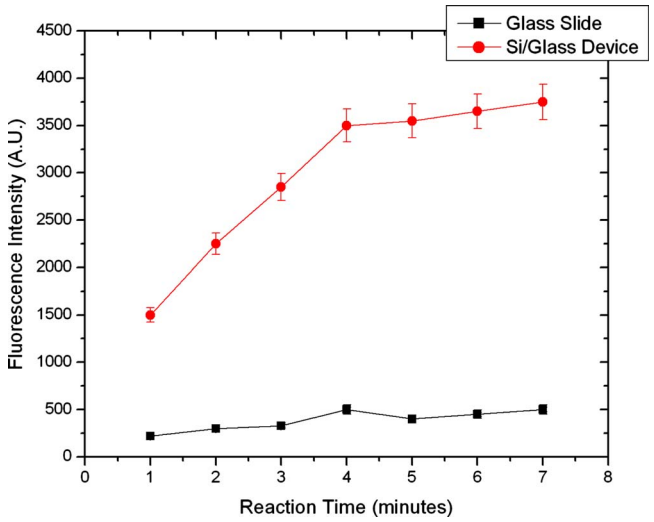

FIG. 5. (Color online) Fluorescence signal intensity (from 0 to 4096) against labeling reaction time between the E_2-BSA-FITC and MCF-7 cancer cells on a glass slide and under flow in the Si/glass microfluidic device

from the LOC is about seven times stronger than that of the labeling signal from the glass slide. The results strongly suggest that the microfluidic approach could achieve a much stronger signal for the same period of incubation. In other words, a microfluidic biochip could enable a much shorter detection time than conventional methods. This is probably due to the continuous flushing and refreshment of the labeling solution, to produce more binding of E_2-BSA-FITC on the cancer cell MCF-7. These are very significant results, because biomicrofludics device and system can therefore be a much more sensitive alternative approach in such manner, compared to conventional glass slides.

IV. CONCLUSION

Current breast cancer diagnostic methods can only detect tumors that have already reached a certain size. By using a microfluidics cellular detection platform, blood or body fluids can be analyzed and the relevant cells can be captured and detected. The device presented here has great potential for early cancer and disease diagnostics, as it benefits from (1) the high sensitivity and selectivity of the well-known immunofluorescence approaches; (2) the rapid labelling efficiency in microfluidic systems (compared to conventional glass slide); (3) the low sample volume needed (microliter range); (4) manufacturability of LOC by 8 in. wafers batch MEMS microfabrication process. Compared to other existing approaches, the filter proposed in this study presents the winning advantage of using widely available current fluorescence microscopes or potential portable optics, without using additional surface chemistry steps or a complicated system integration and high-cost setup. This approach can be extended to the detection of other diseases cells or pathogens.

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