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Portable Coulter counter with vertical through-holes for high-throughput applications

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ABSTRACT

A high-throughput Coulter counter for point-of-care (POC) applications has been developed, which measures the concentration and size distribution of cells in suspension at a single cell resolution. Vertical channels (silicon-based through-holes), instead of conventional planar ones, were employed to achieve a higher density of sensing channels for a high-throughput operation. The crosstalk from the multiple sensing channels was minimized by potentiometric measurement based on a three-electrode configuration, decoupling the neighboring channels electrically. The through-hole array and potentiometric sensing increased throughput up to 8000 cells/s in the prototype (designed with four channels). To secure the sensitivity over a wide range of cell sizes in a limited setting (a portable device powered by a battery power, <5 V), a non-inverting preamplifier with a feedback capacitor was adopted, which amplifies only transient potential change. The enhanced sensitivity by the AC gain extended the measurement range from 6–15 μm to 4–20 μm. The functionality of the prototypes was evaluated by gauging size and concentration of a mixture of microbeads (6, 10, 15 μm) as well as diverse cancer cells spiked in diluted blood. The measurement results were validated with the data obtained from FACS (fluorescence activated cell sorting).

1. Introduction

The blood cell count provides an indication as to a person's general health status and can be used to diagnose a patient's illness and decide on the course of treatment [1–6]. Abnormally high or low counts of each of cell types or the presence/change in the numbers of rare cells in the circulation can be indicative of certain diseases. As specific examples, the number of CD4+ T lymphocytes can be utilized for monitoring AIDS [6] and enumeration of circulating tumor cells can provide useful diagnostic and therapeutic information in cancer patients [4].

Blood cell counting can be completed manually under the microscope using hemocytometers, which allow determining cell counts in a fixed volume (<100 μL) of diluted cell suspension. However, manual counting is labor-intensive, time-consuming and also subject to human errors due to misidentification of cell type [7]. Additionally, it is not even practical for the identification of rare cells in blood owing to their low concentration. Thus, the manual counting methods have been replaced by automatic cell counters such as flow cytometers and Coulter counters.

Flow cytometry is a laser-based analytical methodology employed in multi-parametric analysis such as cell counting and sorting, biomarker and protein detection [8–10]. In addition to the versatility, its capability, high-throughput analysis in a single cell resolution, has made diverse applications in basic research, clinical practice and trials. However, current flow cytometers based on optical measurement are expensive, bulky and complicated, and require specialized expertise for operation, inappropriate to point-of-care (POC) applications [11]. On the other hand, Coulter counters are based on changes in electrical resistance caused by non-conductive cells suspended in an electrolyte [12]. In spite of its limited capability only for counting and sizing, simplicity and cost-effectiveness of the electrical detection method makes it particularly suitable to POC applications.

Though several successful miniaturizations of Coulter counters toward POC applications have been reported [13–16], the Coulter principle inherently suffers from a technical trade-off in the size of microfluidic channel to achieve two important characteristics,

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the throughput and sensitivity. A large size channel is preferred for high-throughput device, while a small size channel can provide better sensitivity. In order to realize high throughput Coulter counter device with the same level of sensitivity, multi-channel configurations have been explored [17]. But such an approach has caused an inter-channel crosstalk; therefore, restricting sensitivity, induced by sharing biasing electrodes from an inlet and an outlet. To address the crosstalk issue in multiple channels, Jagtiani et al. has utilized multiple frequencies for each channel [18] and efficiently suppressed the crosstalk. However, it complicated the circuit design and resulted in limited bandwidth, especially when the number of channels increased. Kim et al. explored a multichannel cytometer for the high-throughput analysis, but their horizontal channel design has limited the scalability to extend to a larger density array [19].

In this work, we described a Coulter counter with multiple vertical through-holes as microfluidic channels and the potentiometric measurement with a three-electrode configuration. The proposed vertical through-hole design preliminarily demonstrated the potential to expand it to a high-density array. In addition, the potentiometric method minimized crosstalk by decoupling signals from the neighboring channels. Especially, a concept of AC gain configuration was employed to amplify only transient potential change to increase sensitivity. We fabricated a prototype with vertical through-holes and electrodes by sandwiching a silicon substrate with two PDMS microchannel layers. Using the devices, the enhanced sensitivity was demonstrated by measuring diverse sized microbeads and cells in a suspension.

2. Methods and materials

2.1. Materials and sample preparation

Sylgard 184 Silicone Elastomer Kit, which was used for the fluidics channel fabrication, was purchased from Ellsworth Adhesives Asia Ltd. Polystyrene beads were purchased from Polysciences, Inc. (Catalog #15714-5 (6 μm), 17136-5 (10 μm), 18328-5 (15 μm)). Phosphate-buffered saline (PBS) was obtained from Sigma–Aldrich (USA). Pluronic F-108 was obtained from Brylchem Pte Ltd, Singapore. Single donor human whole blood was obtained from Innovative Research (MI, USA) (Lot ID: 23 69146B; anticoagulant: K2 EDTA; gender: male; age: 28).

MDA-MB231 (ATCC CRM-HTB-26) is a human breast cancer cell line derived from an adenocarinoma. The cells were cultured in low glucose DMEM (Gibco, cat# 11995-065), 10% fetal bovine serum (Gibco, cat# 16000-044), 2 mM penicillin–streptomycin (Gibco, cat# 15140-122) and 2 mM L-glutamine (Gibco, cat# 25030-081). Cells were cultured at 37 °C in a 5% CO_2 atmosphere in T75 flasks.

MCF-7 cells (American Type Culture Collection, MD, USA) derived from breast adenocarcinoma were cultured in minimum essential media (MEM) (Gibco, cat# 11095-080) supplemented with 10% fetal bovine serum (FBS) (Gibco, cat# 10270106), 1 mM sodium pyruvate (Gibco, cat# 11360-070), 0.1 mM MEM nonessential amino acids (Gibco, cat# 11140-050) at 37 °C in a 5% CO_2 atmosphere in T75 flasks.

All microfluidic channels were coated with Pluronic co-polymer F108 prior to usage [20,21]. The antifouling property of the co-polymer repels proteins and other adsorbent from the surface by steric repulsion, minimizing non-specific binding of microbeads or cells onto the fluidic channel walls.

2.2. Device fabrication

The prototype was fabricated by sandwiching one silicon substrate between two PDMS layers. The three layers were separately prepared. The cast mold for the upper microfluidic channel was fabricated by patterning a 40 μm thick single layer of SU8-2025 photoresist (Microchem) on an 8 in. silicon substrate. On the other hand, the mold for the lower channel was laser-micromachined by cutting a 2 mm thick PMMA sheet (Marga Cipta) to make much lower resistance compared with the upper channel. These molds were replicated by casting and curing liquid PDMS at 65 °C for 4 h. The cured 4 mm thick PDMS layers were sliced into blocks (10 mm × 8 mm), where holes were generated by a biopsy punch (1.1 mm in a diameter) for connecting tubing. The middle layer, silicon substrate was separately fabricated. First, the areas of the vertical through-holes (circular shape, 30 μm of a diameter) were defined by photolithography and the area was dry-etched in a depth of 20 μm with a DRIE (deep reactive ion etcher) from the top surface. Then sensing and shared bias electrode 1 (Ti/Pt electrodes) are defined by lift-off process. Then the wafer was thin down to 400 μm and etched from the back side to form the bigger micro hole with 300 μm diameter. After preparing the three individual components, the two PDMS blocks were aligned and bonded permanently to the silicon substrate by treating with the O_2 plasma on both surfaces. One Pt wire was inserted into the outlet channel for the shared bottom bias electrode 2. The fabrication process of the silicon substrate was illustrated in Fig. S1 in detail.

2.3. FACS measurement

To obtain accurate concentration and size information of cells in suspension, flow cytometry analysis (BD LSRII Flow Cytometry Analyser) was used and compared with our experimental results. Prior to the comparison experiment, the flow cytometer was calibrated with a size calibration kit (Molecular Probes, F-13838) by comparing the forward scatter (FSC) signal with a population of microsphere standards of known diameters. After the calibration, CountBright™ absolute counting beads (Molecular Probes, C36950) consisting of a known concentration of microspheres were added to the sample (cells suspended in PBS with 5% FBS) to determine the cell concentration more accurately. Particle size distribution was obtained using the calibrated forward-scatter signal. The cell concentration can be calculated using the formulae based on the number of bead and cell events according to manufacturers' instructions.

3. Results

3.1. Device design

Fig. 1a illustrates the schematics of a single unit in the potentiometric Coulter counter. A silicon substrate embedded with three electrodes and a vertical through-hole is sandwiched by upper (R_1) and lower (R_2) polydimethylsiloxane (PDMS) microfluidic channel layers (Fig. 1b). A cell suspension introduced from the inlet follows upper, sensing (through-hole), and lower channels sequentially, then is collected from the outlet, as shown in Fig. 1c. To measure the electrical resistance change caused by Coulter principle, a bias voltage is applied over the entire channel by two electrodes near the inlet and the outlet. The voltage at the through-hole is measured at a sensing electrode. The microfluidic channels filled with electrolytes form a series of resistor network so that the voltage divider determines the sensing voltage (V_s),

$$V_s = V_2 + (V_1 - V_2) \cdot \frac{R_0 + R_2}{R_1 + R_0 + R_2}$$

where V_1 and V_2 are the voltages applied to the upper and lower electrodes, and R_0, R_1, and R_2 are the electrical resistance of the sensing, upper, and lower sensing channels, respectively.