A Novel Microscale Coulter Counter for Cell Monitoring and Detection

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ABSTRACT

A novel microscale Coulter counter is designed, fabricated and tested in this paper. The Coulter counter will be used to detect and monitor impedance changes of cells as a function of time in response to different extracellular media. The device can be divided into a passive mixing region, a focusing region using negative dielectrophoretic forces, and a measuring region by multiple electroplated electrode pairs. It consists of SU-8 microchannels, vertical electroplated electrodes and polydimethylsiloxane (PDMS) cover. The devices were tested using both microbeads in saline water and fibroblast cells in phosphate buffered solution. The results show that the proposed microsystem is capable of distinguishing particles of different sizes and monitoring impedance changes of cells.

Keywords: Coulter counter, microfluidic, electroplated electrodes, dielectrophoresis.

1 INTRODUCTION

Cells contain all the information about any living system. Thus, detection and classification of cells is an important aspect of medical research in diagnosis and treatment of diseases at the cellular level. One method for the cell research is to suspend cells and flow them with liquids through a micro-fluidic channel. Electronic particle counters (EPCs) such as the Coulter counter are standard diagnostic devices widely used in laboratory medicine and pathology [1]. An example of these devices is the complete blood count (CBC) which is used to determine the number or proportion of white and red blood cells in the body. EPCs are also used in non-biomedical fields including the manufacture and production of products such as paint, ceramics, glass, and some food [2]. They are also capable of characterizing cells in terms of their size, and other properties that are important to understand the optimization of methods to store them at low temperatures (cryopreservation) [3]. However, traditional Coulter counters have a number of limitations. They are large and are configured to require relatively large sample volumes. This sample volume requirement limits the ability to process samples more rapidly and severely limits measurement of time sensitive cell characteristics. Moreover, these sample size and time constraints are detrimental to accurate dynamic volume measurements.

Miniaturized Coulter counter with various designs using micromachining technology have been demonstrated by several groups [4-12]. These miniaturized Coulter counters provides many advantages such as significantly reduced sample volume, low cost, low power consumption, and portability [4]. However, none of these device are capable of fulfilling our objective in this paper which is to ultimately detect and monitor the cell impedance changes as a function of time after mixing the cells with different extracellular media, because they were designed to measure impedance of cells using one or two electrode pairs and thus may only be used for cell counting purposes and static cell sizing with no mixing involved.

2 PRINCIPLE OF OPERATION AND DESIGN

The proposed Coulter counter consists of three regions: multi-fluidic microchannels with passive mixing of the reagents, negative dielectrophoretic focusing of the cells, and electrical impedance based sensing mechanism. A 3-dimensional schematic of the Coulter counter is shown in figure 1. The micro device is intended to measure the electrical properties of various cell types with diameters ranging from 15-20 μm.

Cells and extracellular media are introduced via two inlets into a Y-shaped channel, and subsequently into the mixing region which consists of serpentine shaped channel that mixes them using chaotic advection and diffusion [13]. The mixed fluids then exit to the focusing region. The electrodes in this region are designed with a ramp-shape that generates a non-uniform AC electric field to focus cells to the center of the microchannel by negative dielectrophoretic (DEP) forces. This focusing region is then connected to the measuring region, and on to the outlet. Our goal is to study how cell properties change over time after they are mixed with a certain extracellular media. This is accomplished by placing multiple electrode pairs along the Coulter channel such that each electrode pair records the impedance of cell at the time it passes through the channel. Thus the impedance changes can be tracked as a function of time across the channel. The distribution of electrodes along the channel was designed such that measurements are

made more frequently at the beginning, during the most transient phase of cell volume change. Traditionally, MEMS based Coulter counters have employed thin films of electrodes patterned on the substrate. This configuration generates non-uniform electric field along the height of the microchannel and thus, those devices generate signal variations if identical particles pass at different heights over the electrodes [14]. In this paper, the MEMS Coulter counter is fabricated with side wall vertical gold electroplated electrodes which will generate uniform E-field over the entire height of the microchannel along the direction perpendicular to the channel.

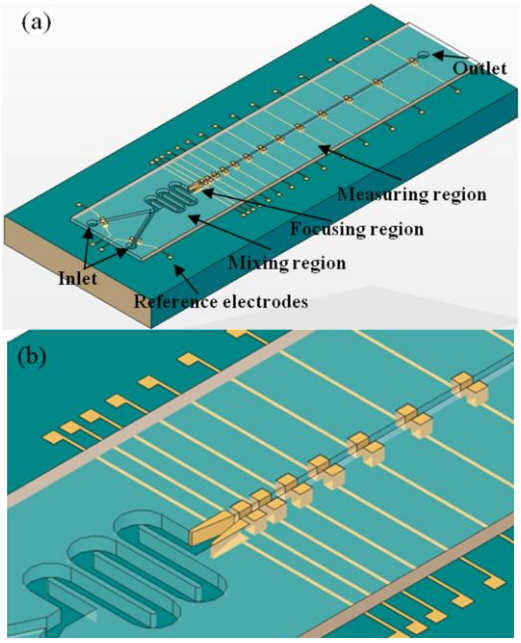

Figure 1: (a) 3-Dimensional schematic of the MEMS Coulter counter; (b) magnified view of the mixing, focusing region and measuring region.

3 FABRICATION

The MEMS Coulter counter is fabricated using metal sputtering, electroplating, surface micromachining, and photolithography on top of a glass substrate using the following sequence as shown in (See figure 2): 1) Glass slides were first cleaned with a piranha solution. 2) A thin layer of SU8 2005 photoresist was spin coated onto the glass slides served as adhesion promoter between SU8 channel and glass substrate. 3) Bilayer of titanium (Ti) and gold (Au) was sputter deposited with thickness of 40 nm and 140 nm, respectively. This layer serves as the seed layer for gold electroplating. Gold layer was patterned and etched to create the electrode traces and bonding pads. 4) An AZ4620 layer with a thickness of 25 μm was spin coated to form the mold for electroplating the electrodes.

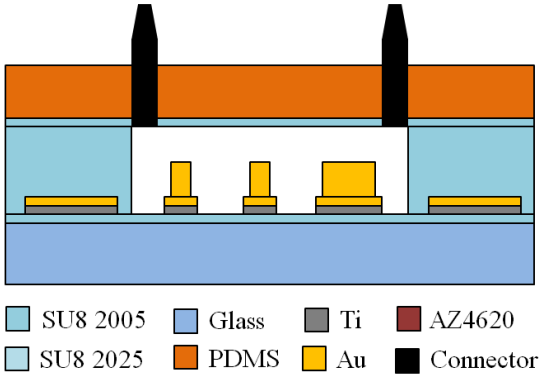

Figure 2: Side view of the Coulter counter fabrication process flow.

The electrodes were created by electroplating gold inside the mold with a thickness around 15 μm, 5) The AZ4620 mold was washed away and the Ti layer was wet etched using gold as a mask layer. 6) The micro channel was defined using SU8 2025 with a thickness of 28 μm. 7) The PDMS slabs (cover) were made and cured to serve as top cover along with fluidic connectors (fluidic inlets and outlets). 8) Oxygen plasma treatment was applied on the

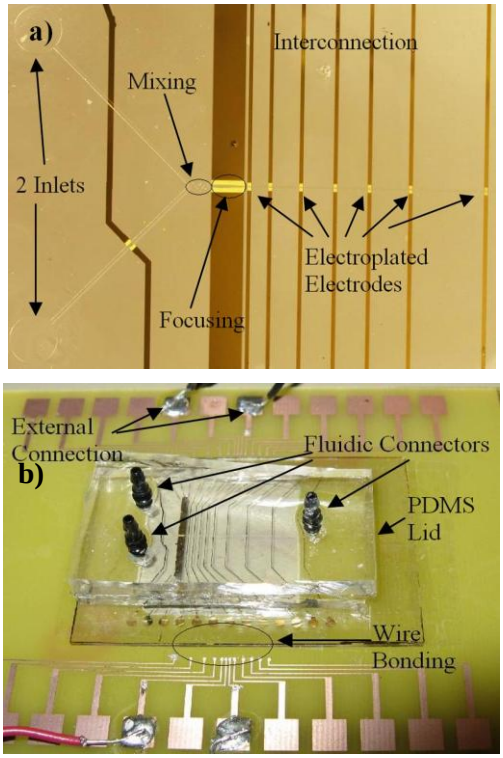

Figure 3: The optical images show: a) the SU8 channel with the mixing region, focusing electrodes, measuring electrodes, and inlets and outlet (not shown) ports, b) a complete fabricated Coulter counter device with PDMS Cover, fluidic connectors, wire bonding and packaging.

PDMS cover in order to change its surface to hydrophilic and then SU8 2005 was spin coated onto it to serve as glue. The oxygen plasma step was used to improve the adhesion between SU-8 and PDMS. 9) The microchannel was then aligned and bonded to PDMS cover. The SU8 on the PDMS cover cross-linked with SU8 channel on the device to form a strong bondage. 10) The device was fixed and wire bonded to PCB for external electrical connections. PDMS was chosen as a cover the channel, due to its advantages including flexibility, ease of fabrication, and transparency. Since PDMS is a rubber like material and it is highly flexible, it conforms to the curvature of the surface where it comes into contact. An optical image, magnified view of the fabricated device, and a complete device with wire bonding, packaging and soldering for external connections are shown in figure 3.

4 TESTING AND RESULTS

4.1 Mixing Testing

The mixing of the fabricated Coulter counter was tested by flowing two fluids with different colors in two streams connected using Y shape junction. The flow rate was controlled by a Harvard Apparatus PHD 2000 syringe pump. The two colors were mixed via chaotic advection and diffusion. The quality of mixing was evaluated by observing color intensity variation of the blue dye using optical microscope at the entrance of the Coulter channel as shown in figure 4.

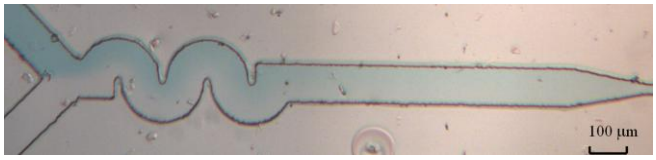

Figure 4: This bottom view optical image displays mixing of two dyes with different colors using passive mixing. The image shows sufficient mixing just before the entrance to the Coulter channel.

4.2 Electrical Testing

An electrical circuit was designed and built in order to measure the resistance changes of microbeads and cells in the measuring region. A resistor and a DC power supply were connected to the electrodes to form a voltage divider. The resistance change in the conductive media, measured by the electrodes can be converted to voltage change. The voltage signal is then passed into a high pass filter in order to block unwanted DC component and then amplified by an instrumentation amplifier and displayed by an oscilloscope. Labview system and data acquisition board (DAQ) USB-6216 (National Instrument, Austin, TX) were later employed in order to record large volume of data from multi-channel for analysis and thus, enable tracking the impedance of same fibroblast cells by multiple pairs of electrodes as they flow through the microchannel.

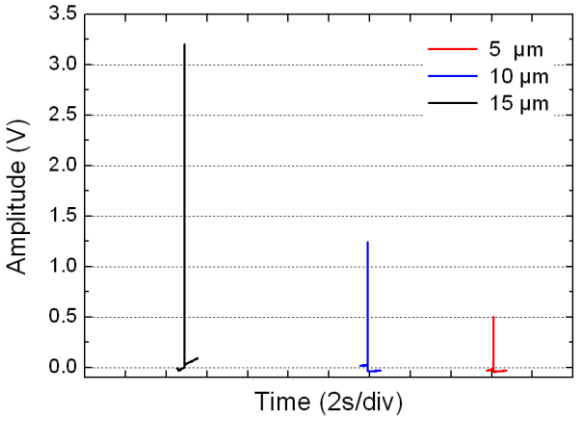

Figure 5: This figure shows voltage pulses with different amplitude which corresponds to microbeads with diameters of 5µm, 10µm & 15µm. The data between pulses were not saved, it was saved only when a pulse is triggered.

The MEMS based Coulter counter performance was tested by injecting saturated (35g/100ml) saline water with nominal 5µm, 10 µm and 15 µm diameter latex microbeads into the microchannel. The measured voltage signals of several microbeads sizes have different amplitude as shown in figure 5. Next the device was tested using fibroblast cells with a diameter of 19 µm. They are suspended in isotonic phosphate buffered solution. Figure 6 shows fibroblast cells passed through one pair of electrode. The difference in signal amplitude indicates that the cell sizes are not equal.

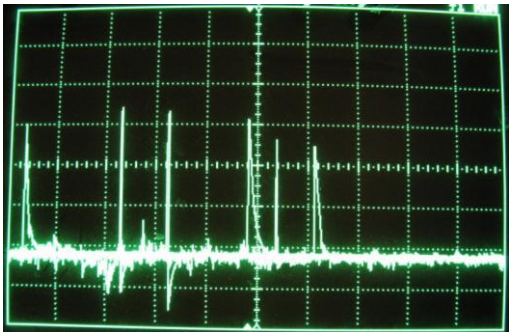

Figure 6: The figure shows several fibroblast cells of different sizes passed through one pair of electrodes. The scale is 0.5V/grid. The negative peaks are caused by DC filter.

5 CONCLUSION

Microscale Coulter counter devices were designed, fabricated and tested in the paper. The device employed

gold electroplated vertical electrodes and SU-8 microchannel. The device will be used for observing impedance changes of cells as a function of time after mixing with different extracellular media. The device uses passive mixing, the phenomenon of negative dielectrophoresis to focus the cells to the center of the channel, and Coulter principle to detect cells based on the change in resistance when they pass through the sensing zone. Both fluidic and electrical testing results using microbeads and fibroblast cells, validate the performance of the device.

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