MEMS based Coulter counter for cell sizing

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ABSTRACT

This paper presents the design and fabrication of MEMS based Coulter counter for monitoring cellular volumetric changes after an exposure to various media. The design consists of a thick SU8 channel which is divided into mixing, focusing, and measuring regions. The mixing region is a serpentine shaped channel, enabling complete mixing of a sample and a reactant before entering the focusing region. The focusing region consists of an electrode pair used to generate AC fields that result in negative dielectrophoretic forces directing cells from all directions to the center of the channel to prevent clogging of the Coulter channel. Finally, the measuring region consists of a channel of width ranging from 20-25 μm, with multiple electrode pairs fabricated using electroplated gold in order to measure the change in impedance at different points along the channel as a cell passes through. This device improves upon existing macro-scale Coulter counter technology by allowing extremely small sample sizes (10^1 compared to 10^5 cells per experiment), an extremely short time frame from the exposure to reactant media to the initial measurement, serial time series measurements of a single cell, and optical microscopic monitoring of the experiment. The design of this chamber will allow for the manufacture of cell specific channel diameters in order to maximize measurement precision for each cell type. This design also eliminates the sheath flow and complex fluid control systems that make conventional cytometers bulky and complicated.

Keywords: Coulter counter, MEMS, Cell sizing

1. INTRODUCTION

Cells are the fundamental unit of any living system. Almost all the information about any organism, from bacteria and algae to plants and animals and humans are stored in individual cells. Any living system is the result of single cell proliferation and cell differentiation processes [1]. Many diseases and disorders have been linked to the deviation of cell concentrations or morphologies from normal values of concentration and morphology. Different aspects of cell research include cell culturing, sampling, trapping, sorting, transportation, lysis and characterization etc [2]. One approach to this research is the growth of cell cultures on chips, i.e., cells adhere to the surface, grow, multiply and can be characterized by electrochemical and physical sensors and analysis methods [3]. Another approach is the micro-fluidic analysis in which the cells are suspended in a liquid and made to flow through channels fabricated in the chip and cell analysis happens as cells traverse through the chip. Micro fluidics based systems implemented by MEMS technology have the advantages of low cost, low energy consumption, low sample consumption and portability [4]. Counting and sizing of particles especially red blood cells is very important for medical diagnostics and other biomedical research applications. A common device for this task is the Coulter counter (Beckman-Coulter, Inc, Fullerton, CA). In the classical Coulter counter, a sample medium suspending the particles to be analyzed is pumped through a small aperture, and the impedance across this aperture is monitored [5]. When a particle passes through the aperture some of the fluid is displaced by the particle and the impedance across the aperture changes proportionally to the volume of the particle. Miniaturization of these macro devices could result in complete lab-on-a-chip systems and may open the door for many other applications including characterization of biological cells, cell handling, cell analysis and cell sorting.

For these systems to work efficiently, it is important to confine the sample to the center of the channel as a small well-defined volume in order to eliminate clogging the Coulter channel entrance [6]. Hydrodynamic focusing has been widely used to focus particles into a tight stream by two sheath fluid flows on either side [7]. This technique requires complex systems for controlling the proper flow rates and mixing of the sheath and sample flows. Moreover, there is possibility of fluid diffusion. In addition a reservoir is required for the sheath flow medium that must be kept free of dust and bacteria

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[8]. Several attempts have been made to overcome these drawbacks. Huh *et al.*. proposed to use air as a sheath fluid, which eliminates the complex maintenance of the liquid reservoirs and thereby reducing the overall weight and volume of the entire system [9]. Schrum *et al.* proposed to use electrokinetic flow rather than a mechanical pump to drive and control the sheath flow [10]. Many other alternatives have been explored to replace hydrodynamic focusing in micro-fluidic devices. One such alternative is the use of electric fields to move, rotate, manipulate or sort different types and sizes of cells. In this paper we use the phenomenon of negative dielectrophoresis to focus the cells to the center of the channel and use the Coulter principle for counting and sizing of different cell types.

2. Theoretical background

2.1 Mixing

Mixing of reagents is an important aspect in this study, as the experiments are designed to monitor cellular volume after an abrupt change in the composition of the extra-cellular solution. Higher mixing efficiency is always required to increase the reaction speed and improve the sensitivity of these systems.

All cellular membranes are freely permeable to water and the net movements of water between the cells and their surrounding interstitial fluid are determined by the osmolalities of these two compartments [11]. If the osmolality of the interstitial fluid decreases, fluid must enter the cells and the cell volume increases. On the other hand if the osmolality of the interstitial fluid increases water must leave the cells and hence cell volume decreases. Thus the basic physiological mechanisms that determine and control the osmolality of the extra-cellular fluid affect the cellular volume. The micro-fluidic system discussed here allows the mixing of two different media in a Y-shaped channel. To enhance the mixing efficiency of the device, a passive mixing using serpentine shaped channel geometry is designed. This will increase the interfacial area of contacts between the two fluids and generates chaotic advection in these fluids.

2.2 Focusing

The phenomenon of negative dielectrophoresis is used to focus the cells to the center of the channel. Dielectrophoresis (DEP) is the translational motion of a particle in a suspending medium under the influence of a nonuniform AC electric field [12]. The application of a nonuniform AC electric field induces a dipole moment in a charge-neutral particle, causing the particle to move toward a region of maximum or minimum electric field strength. The driving direction depends on the polarizability of the particle compared with that of the medium surrounding the particle and the frequency of the applied electric field. If the polarizability of the particle is more than that of the surrounding medium, it is called positive dielectrophoresis and the particle tends to move towards the region of high field strength. On the other hand if the particle is less polarizable than the surrounding medium the phenomenon is termed as negative dielectrophoresis and the particle tends to move towards the region of low field strength. The phenomena of positive and negative dielectrophoresis on particles under the influence of applied electric field is shown in Figure 1. The dielectrophoretic force F_{DE} acting on a spherical particle of radius 'r', subjected to non-uniform electric field 'E' is given by:

$$F_{DE} = 2\pi\varepsilon_m r^3 \,\mathrm{Re}[f(\varepsilon_p^*, \varepsilon_m^*)]\nabla\vec{E}_{rms}^2 \tag{1}$$

For a spherical homogenous particle f ($\varepsilon_p^*, \varepsilon_m^*$) is referred to as the Clausius-Mossotti factor (C-M factor) [13]

$$f(\varepsilon_p^*, \varepsilon_m^*) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} = \frac{\varepsilon_p - \varepsilon_m - j\dfrac{(\sigma_p - \sigma_m)}{\omega}}{\varepsilon_p + 2\varepsilon_m - j\dfrac{(\sigma_p + 2\sigma_m)}{\omega}} \tag{2}$$

where, ε_p^* and ε_m^* are the complex permitivities of the particle and medium, respectively, ω is the angular frequency, σ_m is the conductivity of the medium, and σ_p is the conductivity of the particle. From Equation-1 it is clear that the dielectrophoretic force depends not only on the properties of the particles and the medium in which they are suspended but also on the amplitude of the applied field. The force is attractive or repulsive based on the sign of the real part of the C-M factor which in turn is a function of the frequency of applied field $\mathrm{Re}[f(\varepsilon_p^*, \varepsilon_m^*)] = 0$ at the cross over frequency, i.e. the particle is subjected to zero dielectrophoretic force. At frequencies above the crossover frequency $\mathrm{Re}[f(\varepsilon_p^*, \varepsilon_m^*)]$

is positive i.e., positive dielectrophoresis and for frequencies below the crossover frequency Re[f(ε_p^*, ε_m^*)] is negative i.e., negative dielectrop

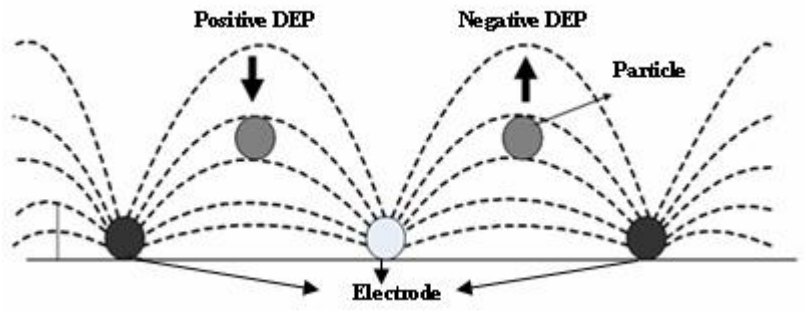

Figure 1 Schematic diagram showing the Positive and Negative Dielectrophoretic forces on particles subjected to non-uniform electric fields.

For the dielectrophoretic focusing of the cells, vertical electrodes on the sidewalls of the micro-channel have been fabricated. This configuration of electrodes results in non-uniform electric field over the entire height of the channel. An impedance analyzer will be used to measure the impedance across each electrode. One advantage is that they can measure impedance (inductance, capacitance, and resistance) at spot frequencies or across a range of frequencies. Thus impedance measurements can be performed at multiple frequencies and the response of cells to different frequencies can be studied. The choice of frequencies should be such that the particles are subjected to a negative dielectrophoretic force which directs them to the center of the channel, thus aligning them into a thin stream at the center. Thus, to achieve the optimum dielectrophoretic focusing the device should be operated in conditions where the negative dielectrophoretic force is the maximum. This can be possible by operating at frequencies below the cross over value. Also, the value of applied voltages should be less than the threshold for damaging the cells.

3. Design

A schematic view of the Coulter counter is shown in Figure 2. In design, The micro-Coulter counter structure can be used to measure the mechanical properties and degradation of various types of cell such as red blood cells (6.6 -7.5 µm diameter), sperm cells (1-3 µm diameter, ~100 µm length), stem cells (8-12 µm diameter), oocytes (70-100 µm diameter) and other cell types of diameter ranging from 15-20 µm in diameter. It consists of microchannel with a depth of 25 µm fabricated using SU8. This channel is divided into mixing, focusing, and measurement regions (Figure 2). The mixing region is a serpentine shaped channel, enabling complete mixing of a sample and a reactant before entering the focusing region. The focusing region consists of an electrode pair used for negative dielectrophoretic focusing of the cells, designed to prevent clogging of the channel. Finally, the measuring region consists of a channel of width ranging from 20-25 µm, with multiple electrode pairs fabricated using electroplated

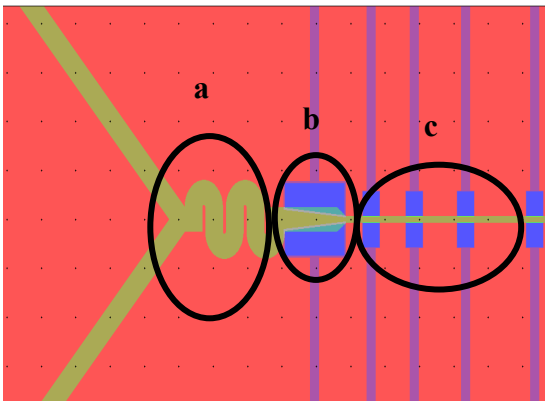

Figure 2. Schematic view of the Coulter counter, a) mixing Region. b) focusing Region. c) measuring Region.

gold in order to measure the change in impedance at multiple points along the channel as a cell passes through. By the Coulter principle, this change in impedance is directly proportional to the volume of the cell.

4. Fabrication

The micro Coulter counter is fabricated using metal sputtering, electroplating, surface micromachining, and photolithography on microscopic glass slides as shown in Figure 3. The device fabrication steps are described as follows. Initially, the glass slides were cleaned in a piranha solution (H_2SO_4: H_2O_2, 3:1) for 3 min and then washed thoroughly with DI water. Next, two layers of titanium (Ti) and gold (Au) were sputter deposited with a thickness of 40 nm and 140 nm to serve

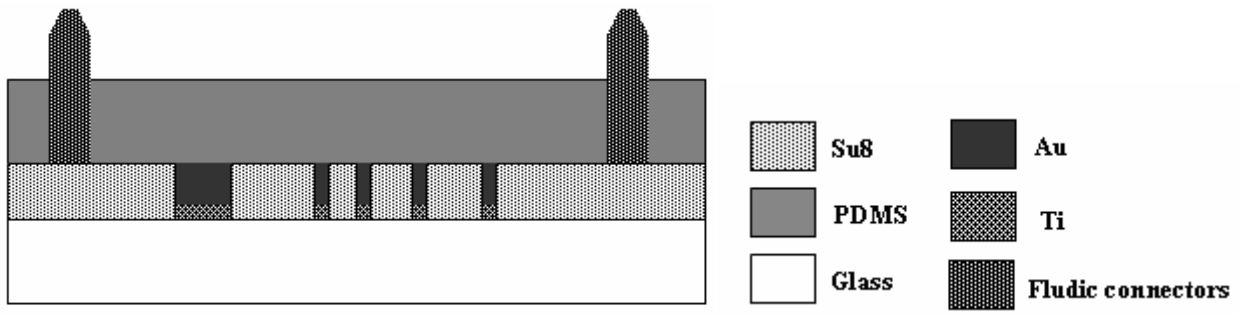

Figure 3. Side view of the Coulter counter fabrication.

as the seed layer for electroplating the electrodes. Next a mold was created for electroplating the electrodes using a thick AZ4620 (Clariant) photoresist layer with a thickness of 16μm. The electrodes were created by electroplating gold inside the mold with a thickness of 12 μm. The electroplating was performed using the electrode mask and the device was dipped into gold electroplating solution (Technic gold 25 ES) to plate the gold electrodes at well controlled temperatures, stir rates and current densities (55°C and 300rpm). Next, the glass slides were cleaned using acetone, methanol and then rinsed with DI water. The electrode traces and bonding pad were patterned by etching Au using KI/ I_2 solution. The Ti layer is used as an adhesion layer for gold. The Ti layer was etched in diluted hydrochloric acid (HCl). The next step was to define the channel using SU8. An optical image of the device without the top cover is shown in Figure 4. A thick layer of SU8-2025 was patterned using the channel mask and then washed with IPA and hard baked at 120°C for 30min. A connector mask was used to mark the positions of the fluidic connectors on the top cover microscopic glass slide. Fluidic connectors (from Harvard Apparatus) were temporarily fixed on the marked positions using quick glue. Once the fluidic connectors were properly fixed, these glass slides were placed in a petri dish for the PDMS processing.

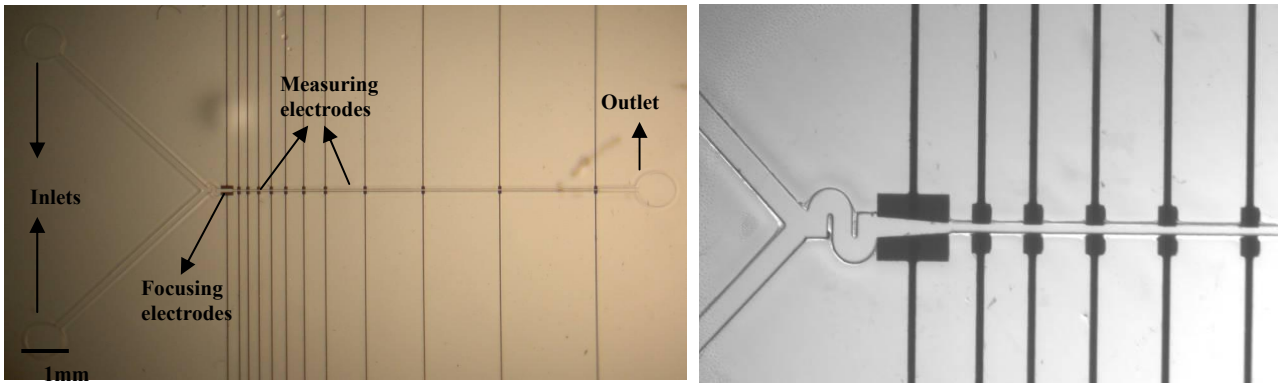

Figure 4. Microscopic image of the Coulter counter showing (a) the SU8 channel with the focusing electrodes, measuring electrodes, inlet and outlet ports, (b) a magnified view of the device.

A 10:1 mixture of PDMS base and curing agent was prepared and poured in a Petri dish and left overnight to cure. The cured PDMS slab along with the embedded fluidic connectors was then peeled off from the Petri dish and cut into cubes

of desired sizes and was used to seal the channel. The device was then packaged on a copper coated PCB board patterned with the bonding pads for external connections. Sample media flows through tubes fitted to the device via connectors housed in the PDMS slab. An image of the fabricated device with the PDMS cover and the fluidic connectors is shown in Figure 5. The complete device with wire bonding, packaging and soldering for external connections is shown in Figure 6.

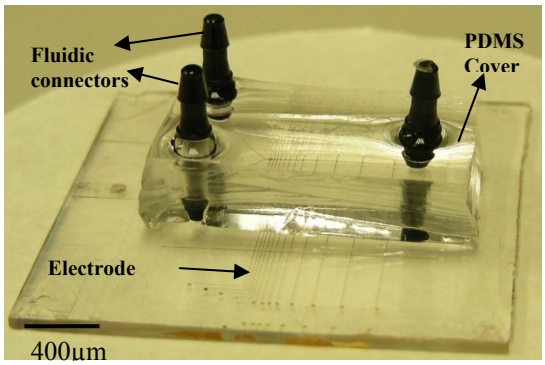

Figure 5. Digital photo of the complete fabricated Coulter counter with the PDMS cover and fluidic connectors.

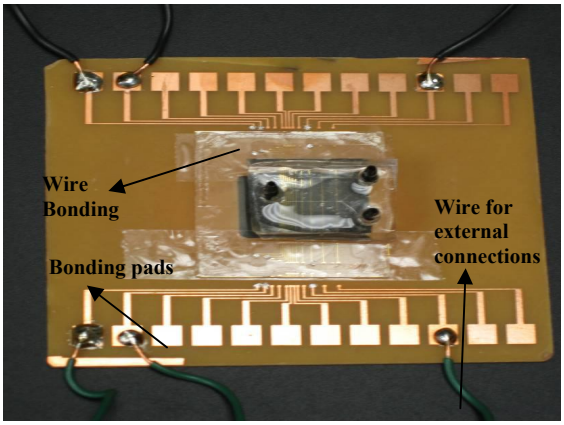

Figure 6. A digital photo of the Coulter counter with wire bonding and packaging.

5. Testing and results

5.1 Fluid flow in the channel

The fluid flow was tested by flowing ethanol through the channel by using a syringe pump. Several experiments were conducted to improve the device performance using the setup shown in Figure 7. Polydimethylsiloxane (PDMS) was chosen as a cover for the channel, due to its advantages such as flexibility, biocompatibility, and 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 it comes into contact with. The PDMS cover was prepared using a mold, and fluidic connectors fixed on a glass slide

The PDMS mixture was prepared and poured in the Petri dish and left at room temperature for 24 hrs overnight or in an oven at 90°C for 20 min. The cured PDMS is later cut into cubes and peeled off from the mold. The PDMS slab was then spin coated with a thin layer of SU8 2005 (about 5 µm). If SU8 is directly spin coated on the PDMS slab, it will shrink to the center after baking. A hydrophilic PDMS surface can ameliorate this problem, and one method to make the surface of PDMS hydrophilic is to treat it with oxygen plasma. In this case, SU8 spreads uniformly and adheres to the treated PDMS surface after baking. The PDMS slab with spun SU8 was baked on a hotplate at 90°C for 10min.

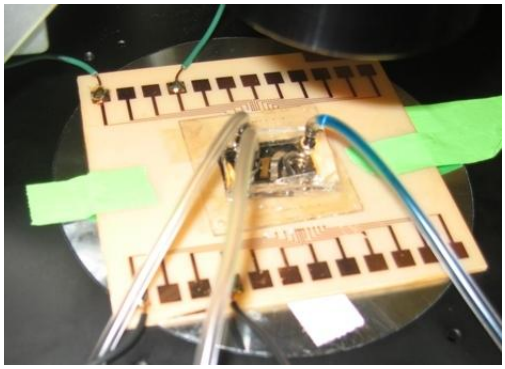

Figure 7 Setup for fluidic testing of the Coulter counter.

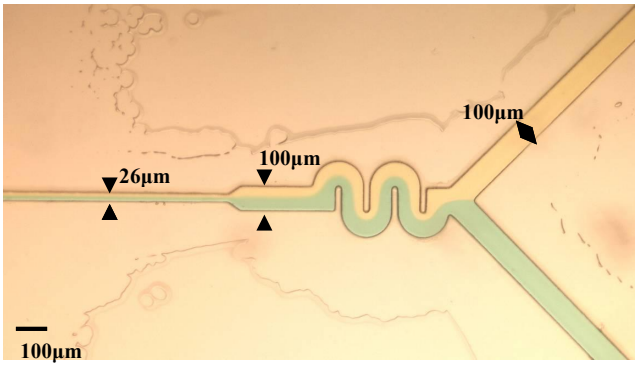

Figure 8. Fluid flow in the Coulter counter with PDMS slab sealing the channel. No mixing seen at high flow rates.

The PDMS slab was then aligned to the channel-device and the whole device was baked at 65°C for 10 min while applying enough pressure by placing a weight on top of it. The device is then exposed to UV light for 40 sec and later hard baked at 120°C for 20 min to crosslink the SU8 layer and to glue the PDMS slab to the channel. The connectors were then placed in the holes on the PDMS cover and PDMS mixture was poured around them and allowed to cure to make sure they are properly fixed in position. This device was tested for fluid flow as shown in Figure 8.

5.2 Mixing of fluids in the channel

Mixing of two reagents in the channel is essential as the experiments are designed to monitor cellular volume after a change in extra-cellular media contents. To test if mixing in the channel is efficient, two fluids with different colors i.e., yellow and blue dyed fluids flowing through the channel should theoretically result in a green colored mixture. Thus, blue and yellow dyed ethanol were perfused through the channel at equal flow rates by using two syringe pumps. At high flow rates (~ 10 µl/min) it was seen that there is no mixing of fluids in the channel as shown in Figure 8.

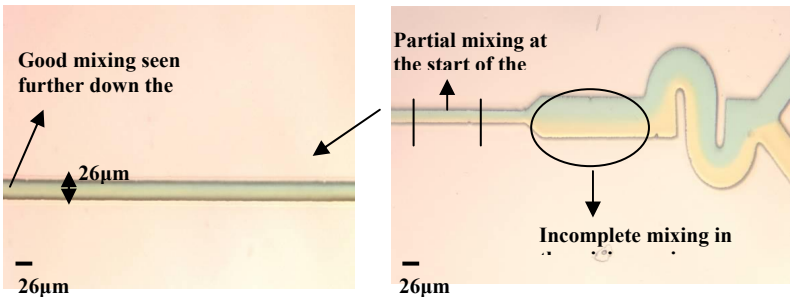

Figure 9 Mixing at different flow rates.

When the flow rate of one of the fluids was reduced to 2 μl/min and the flow rate of the other was 3 μl/min, it was seen that although there is not a complete mixing of the fluids in the mixing region of the channel, there is good amount of mixing in the measuring region of the channel as shown in Figure 9 (left). Further optimization of mixing region design should enhance mixing at higher flow rates. Thus, the device is successfully fabricated with no fluid leakage in the channel and also a good mixing of reagents in the channel was achieved.

6. Conclusion

A micro-scale Coulter counter device has been fabricated which will be used for counting and sizing different types and sizes of cells. This device presented here uses 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. The design has been optimized to prevent fluid leakage, which is a major issue with most micro fluidic devices.

7. References

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