Lab-on-chip Microfluidic System for Single Cell Mass Measurement

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***Abstract*— In this paper, we presented a lab-on-chip microfluidic system for single cell mass measurement. Single cell mass has been related with drag force exerted on cell and Newton law of motion. Drag force has been generated using pressure driven syringe micropump. Motions of the cells were measured using optical observation under an inverted microscope. We have calibrated the approach using known mass of polystyrene microbeads with a diameter of 5.2 µm. Our experimental results showed that, mass of single microbead is 88.9 fg, which is very close to the theoretical mass (77.3 fg) of the particle. From which it is believed, our approach is suitable for single particle's mass measurement. We used *Saccharomyces cerevisiae* baker's yeast cell as a cell sample. Yeast cells have been cultured in our laboratory using YPD medium. For the diameter of 4 µm cell, mass of single cell was measured as 1.9 pg which is very consistent with previously reported single yeast cell mass (1-3 pg).**

Keywords-microfluidic device; drag force; cell culture; single yeast cell mass.

I. INTRODUCTION

Micro electro mechanical systems (MEMS) provide an excellent platform to analyse single cell mechanics often known as lab-on-chip microfluidics device [1-5]. Studies on single cell mechanics acquire a great interest of scientists as cell mechanics can be related to the early diagnosis of disease through single cell surgery and cell wall stiffness [6]. Cell mechanics consist of (but not limited to) cell wall strength, cell mass, density and volume at different phase of cell growth cycle. Among them, single cell mass is an important parameter as cell mass depends on the synthesis of proteins, DNA replication, cell wall stiffness, cell cytoplasm density, cell growth, ribosome and other analogous of organisms [7].

Chronic diseases like cancer, tumor affect intracellular physiological properties of cells [8], subsequently cell mass and density will be changed as well [9-10]. For example, in a tumor infected cell, integrity of DNA faces continuous challenges and genomic instability occurs to the chromosome's structure. Inevitably, this will cause severe change to DNA replication, cytoplasm density and cell volume which ultimately leads to the changes in single cell mass. In this condition of cell, if we could determine the mass of a single cell, we will be able to differentiate the unhealthy cell from healthy cells by investigating single cell mass property. Single cell mass has also a great contribution in terms of rapid identification of dangerous virus and bacteria. As a result, we strongly believe that studying single cell mass and its measurement techniques will enhance our knowledge of cell mechanics.

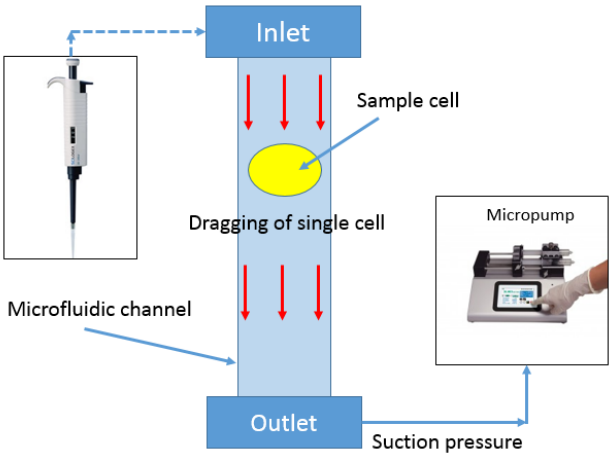

Figure 1. Microfluidics channel for single cell mass measurement. Suction pressure has been applied to outlet of the channel which generate drag to the cell. This drag force has been related with Newton force of motion to measure the mass of single cell.

Recently, lab-on-chip suspended microchannel was developed to measure dry cell mass accurately [4], [11]. The sensor has been optimized to obtain a high aspect ratio sensor's geometry which enables the sensor to measure object mass in femtogram (fg) range [12]. Even though, suspended microchannel resonator has a great contribution to the advancements of single cell mass measurement techniques, yet this method is limited to dry cell only [2] and cell stiffness data remained elusive. As a consequence, 'living cantilever arrays' was proposed to measure adherent cell mass [7]. In this method, cantilevers were submerged into the L-15 growth medium and cells were cultured. Hence, live adherent cell mass was measured using cantilever arrays mass measurement sensor. However, the cantilevers sensor has non-uniform mass sensing ability [13], as a result accuracy depends on the cell position merely and measurement error could be up to 40% [7]. Apart from these,

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suspended microchannel/cantilever require a very complex fabrication procedures with sophisticated state of art fabrication facilities. Our proposed approach is novel, much simple in fabrication and its ease the tedious experimental procedures for single cell mass measurement.

We are proposing single cell mass measurement approach from the single cell flow inside microfluidic channel. We have applied Newton drag force on the cell using pressure driven micropump by which cells are accelerated. Fig. 1 illustrated the concept. Drag force has been calculated using (1)

$$F_d = \frac{1}{2}\rho v^2 C_d A \quad (1)$$

where, F_d is the drag force, ρ is the liquid density, v is the cell velocity, C_d and A is the cell's drag co-efficient and area respectively. Among the above parameters, velocity and cell's area can be measured using optical observation under microscope in the known density of liquid. Drag coefficient is a dimensionless parameter, depends on the particle's geometrical shape. For the microfluidics channel, Reynolds number is very low (Re<<1) i.e. the flow is fully laminar [14]. At this low Reynolds number drag coefficient has been suggested for spherical object as 0.1-0.5 [15]. From the above mention information we can measured the applied drag force on the cell. Due to the exerted drag force on the cell, cell will be accelerated and will move forward. This acceleration can be related with Newton second law of motion as illustrated in (2)

$$F = ma \quad (2)$$

where, F is the exerted force, m is the mass of cell and a is acceleration due to the force. Equating (1) and (2) mass of the cell/particle can be measured. The accuracy of the results depend on the careful measurement of the parameters like liquid density, particle velocity, acceleration. We strongly believe that, this approach of single cell mass measurement will contribute to the knowledge of biomedical engineering significantly.

II. Fabrication of the Microfluidic Chip

We have fabricated the microfluidic channel using polydimethylsiloxane (PDMS, SILPOT 184, Dow Corning Corp.) material. PDMS is transparent and biocompatible material, which makes this material very popular for biomedical applications. Fig. 2 (a)-(d) described the detail of the procedures that we used for fabrication. We developed the master mold on silicon surface using soft lithography technique. Fig. 2(a) showed the schematic of master mold. Width and depth of the channel is 15 µm and 10 µm respectively. PDMS material was poured on the mold surface and treated for 24 h at the room temperature. After 24 h, PDMS was dried and replica was generated for microfluidics system. Then the PDMS was pilled of from the surface and drilled the inlet and outlet. Diameter of the inlet and outlet are 1 mm. Finally the PDMS chip is ready to use [(Fig. 2(c)]. At Fig. 2(d) the PDMS is placed on the glass surface and ready for the experiments. Fig. 3 illustrated more details about the fabricated microfluidic chip.

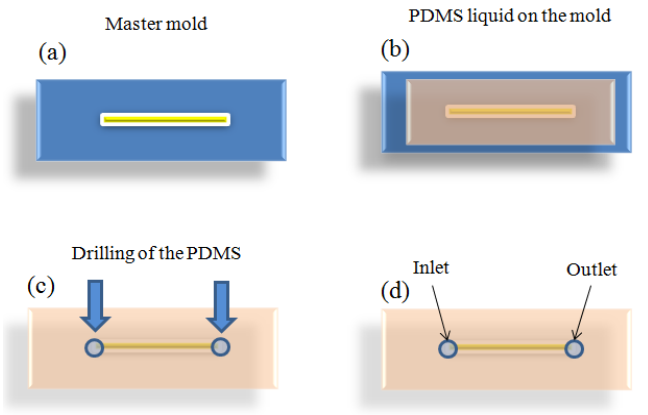

Figure 2. Fabrication procedures of the PDMS microfluidic channel. (a) Master mold after soft photolithography. (b) PDMS liquid layer on the master mold. (c) Dried PDMS structure and drilling of the channel. (d) Inlet and outlet of the microfluidics channel. Microfluidics system was fabricated at Micro Nano System Engineering Laboratory, Nagoya University, Japan.

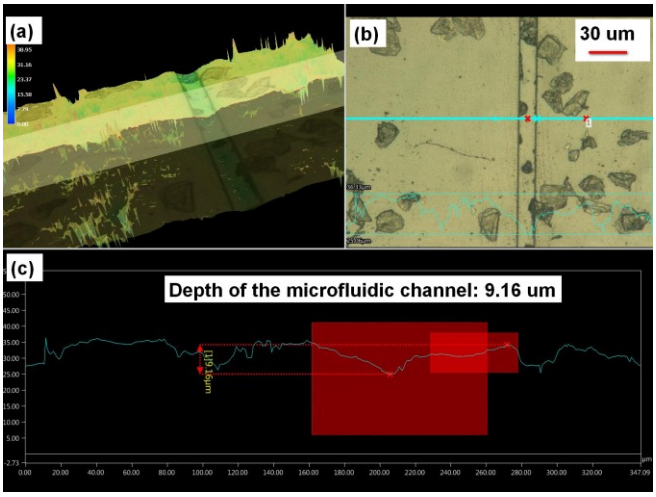

Figure 3. Fabricated PDMS microlfuidics system. (a) 3D view of the microlfuidics channel. (b) Top view of the channel. (c) Depth of the microfluidics channel is 9.6 µm. The images have been captured using Keyance Digital Microscope: VHX 5000.

III. Calibration of the Microfluidic Chip for Single Cell Mass Measurement

We have calibrated PDMS microfluidic mass measurement system using commercially available polystyrene microbeads. Spherotech PP-50-10 polystyrene particle was used to verify the mass measurement approach. As obtain from the particle datasheet, average diameter of the particle is 5.2 µm, volume 73.6 μm^3 and the density of the polystyrene is 1050 kg/m^3. From these data, theoretical mass of the single microbead was calculated as 77.3 fg (f = E-15). After calibrating our proposed method, we compared our experimented result with the theoretical value of single particle mass. To measure single particle mass we need two major procedures; measure the acceleration of the particle and calculate the drag force exerted on the particle.

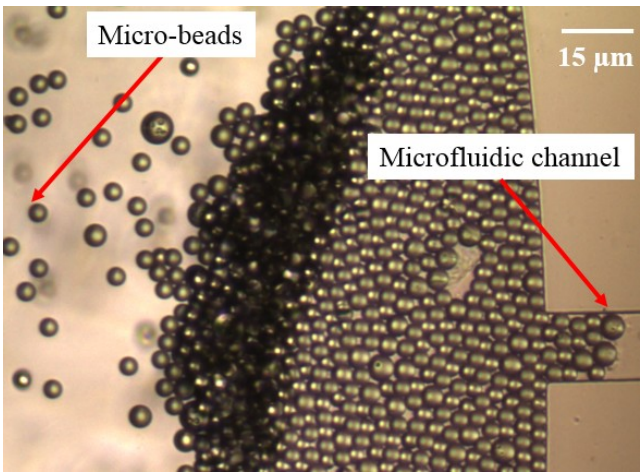

Figure 4. Postyrene microbeads inisde microfluidic channel. Average diameter of the each bead is 5.2 µm.

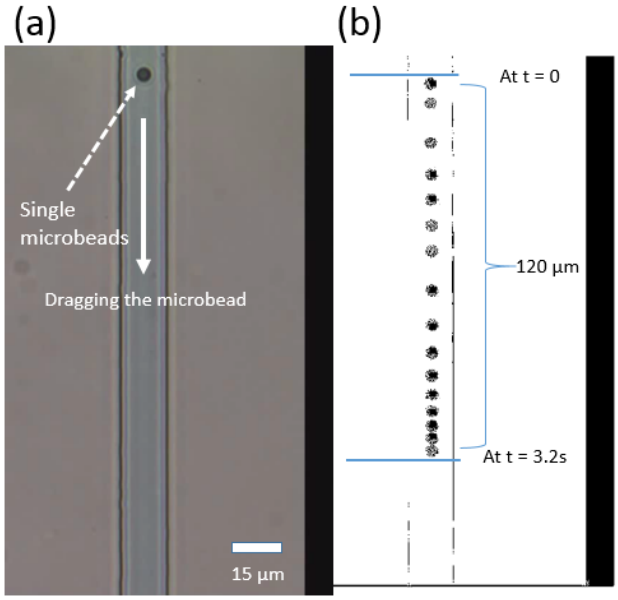

Figure 5. (a) Image captured from inverted microscope, shows that single microbead is flowing through the microfluidic channel due to drag force exerted on cell from the pressure driven micropump. (b) Time lapse image of the particle flow for 3.2 sec. Distance covered in this time is 120 µm. Average velocity of the particle is 37.5 µm/s.

A. Measuring the velocity and acceleration of the particle

Measuring the velocity and acceleration of the particle/microbead is the most challenging part of this experiment. The experiment was conducted under inverted microscope, IX73, Olympus. Legato 200, Syringe micropump (KdScientific) was used to control the particle flow inside microfluidic channel.

We have divided particle's velocity and acceleration measurement procedures into 4 steps. Firstly, inject the sample into inlet and provide suction pressure at the outlet. The suction pressure will direct the microbeads at the gate of microchannel and microbeads will tend to flow. Secondly, apply suction pressure in the outlet. We have applied withdraw flow rate of 50 µl/min for 10 mints. This flow rate generated a pressure of approximately higher than 10 KPa at the outlet. Pressure was measured by T-tube connection of MPX10 Piezoresistive pressure sensor, Freescale Semiconductor.

According to the Hagen-Poiseuille law, pressure inside microfluidics channel drop significantly [16]. This pressure drop will cause the particles to be flown through the microfluidics channel from inlet towards outlet [17]. Fig. 4 showed that microbeads are tending to flow inside microfluidic channel. Initially, single particles move very fast due to viscous flow of water. But at the saturation stage when the channel is full with water we were able to observe single particle flow clearly [Fig. 5 (a)]. At third step, when single particles are moving through the microfluidic channel, we have recorded the flow of water using FastStone video recorder software for 3.2 seconds (recording time is flexible). Finally, at the forth step, we have splitted the 3.2 seconds video in every 0.2 seconds and develop time lapse using our own developed Matlab Simulink Coding [Fig. 5(b)]. Fig. 5(b) illustrated that, total distance covered in this time lapse was 120 µm in approximately 3.2 seconds with the average velocity of 37.5 µm/s. The average acceleration of the particle was measured as 18.56 µm/s^2.

B. Characterizing the mass of single micro particle

Once, acceleration of the particle has been measured, we need to measure the force which generated this acceleration. This force is equal to the drag force exerted on the particle. Drag force can be calculated using (1) if the parameters are known. For this experiment, we used water as a flow medium which density is 1000 kg/m^3. Area (πr^2) for a 5.2 µm diameter of spherical single microbead is 21.2 µm^2, velocity of the moving particle was measured as 37.5 µm/s and dimensionless parameter drag coefficient for the spherical particle is 0.1 [15].

Using these parameters, drag force was measured as 1.65E-18 N which generated an acceleration of 18.56 µm/s^2 to the particle. At this stage, pressure driven drag force is equal to the force of Newton second law of motion which depends on the particle mass [18]–[20]. Now using Eq. (1), we measured the mass of single polystyrene particle as 88.9 fg which is very close to the theoretical mass of the polystyrene particle (77.3 fg). From these calibration results, we can envisage that proposed LOC microfluidics system is suitable for single particle/cell mass measurement. To measure single cell mass, instead of using animal cells we have used baker yeast cell as a sample cell for mass measurement.

IV. Mass of Single Yeast Cell

Saccharomyces cerevisiae baker's yeast cell was used as a sample of cell. Yeast cells are eukaryotic microorganisms in the classification of fungi [9]. Yeast cell has been widely used a sample cell for disease diagnosis like tumor, caner, Parkinson's etc. Yeast cell also been used to investigate the mechanical properties of cell like elastic modulus [21], cell wall strength [6], cell cytoplasm density and conductivity etc. The geometrical shape of yeast cell spherical and diameter varied from 3-7 µm [22]. In this work, we have cultured yeast cell in our laboratory and measure the mass of a single yeast cell with a diameter of 4 µm.

A. Yeast cell culturing

Yeast cell has been cultured using conventional cell culturing methods. Yeast growth medium/agar has been developed using YPD and sucrose in a ratio of 1:1 in one liter of water. The mixture was then shacked for few minutes to ensure the proper dilution of the YPD and sucrose. Later on, yeast powder was inserted inside the growth medium. The mixture of the YPD, sucrose and the yeast powder was than kept on the sunlight for 2 hours by which yeast cell has been culture properly. From the cultured yeast cell, we have pipetted some of the cells in to petri dish and observed under microscope. Fig. 6 shows the cultured yeast in our laboratory. Yeast cells has been submerged into blue dye mixed water to get a better view under inverted microscope.

B. Mass of single yeast cell

To measure the mass of single yeast cell we have used similar approach as we explained in the calibration section. Firstly we measured the velocity and acceleration of the moving cell then we measured the force required to accelerate the cell. Initially, cultured yeast cells were diluted with water and then injected into the microfluidics channel. Using micropump we applied suction pressure inside the microfluidic channel. Suction pressure generated drag force on single cells and cells started to flow. For this particular measurement we have selected a well visible cell which diameter was 4.4 µm and the volume of the cell was 44.5 μm^3. Due to the applied suction pressure cells were covered a displacement of 107 µm in 5.5 s (Fig. 7). Displacement was measured using an image analyzing software (Image J), developed by the National Institute of Health, Japan. The initial velocity of the yeast cell was 19.45 µm/s. Drag coefficient of the yeast cell is 0.1 [23] and the density of the yeast cell cultured medium was measured as 1180 kg/m^3 using our weight balance equipment (Shimadzu, *ATX224)*. From these parameters using Eq. 3.1 we measured the drag force to move the cell as 3.39 E-19 N.

Once the cells start to flow we stopped applied pressure and deceleration occurred to the cell. After 5.5 s the velocity of the yeast cell was 18.5 µm/s. As a result deceleration occurred with the magnitude of 0.17 $\mu m/s^2$. Now by equating Eq. 3.1 and 3.2 we measured the mass of single yeast cell as 1.9 pg. This result is very much consistent with the cell mass measurement using suspended microchannel resonator (SMR). Previously reported mass of an adult (4 µm diameter) yeast cell varied from 1-3 pg [12].

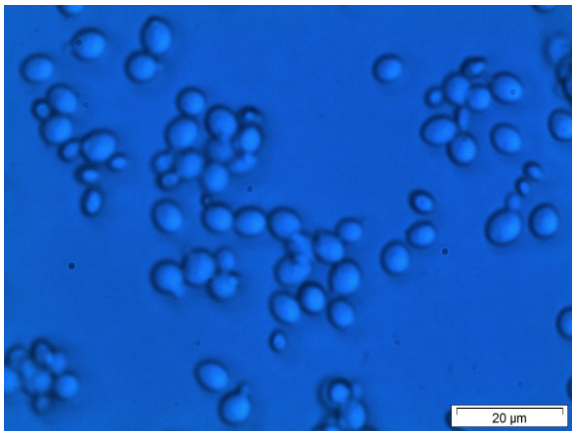

Figure 6. Cultured Baker's yeast (Saccharomyces cerevisiae) yeast cell in our laboratorty. The diameter of cell varied from 2-7 µm.

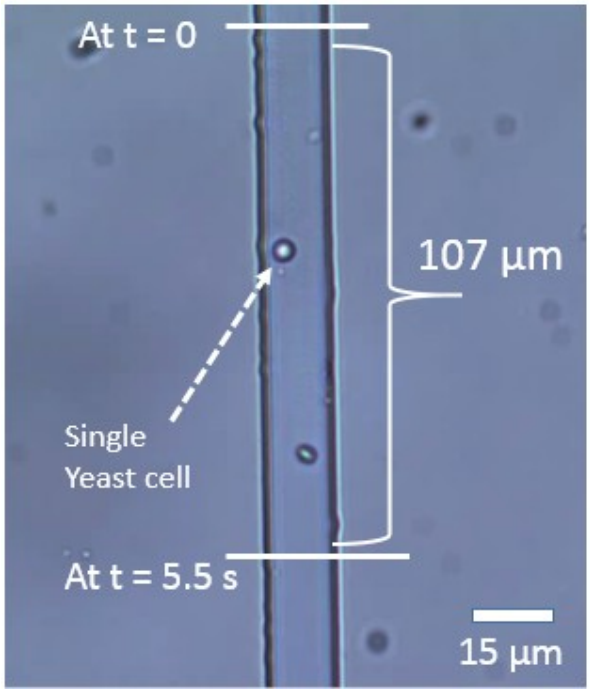

Figure 7. Single yeast cell flow through microfluidic channel. Cell covers a distance of 107 µm in 5.5 sec at the average velocity of 19.45 µm/s.

V. Conclusion

A novel method for single cell mass measurement has been successfully presented in this paper. A microfluidic channel was fabricated on a PDMS chip. Single micro particle/cell was flown through the microfluidic channel. drag force and Newton second law of motion has been applied to measure single cell mass. Proposed method has been calibrated using known mass of commercially available polystyrene microbeads. We have also measured the mass of single baker's yeast cell. Single yeast cell mass was measured as 1.9 pg. In future, this approach can be applied to measure mass of human cell and perhaps it may provide new tools for disease diagnosis through the variation of single cell mass property of identical cells at different health conditions.

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