Chapter

Microfluidic Device for Single Cell Impedance Characterization

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Abstract

Detection of single particle has emerged as a noninvasive technique for diagnostic and prognostic patients with cancer suspected. Microfluidic impedance cytometry has been utilized to detect and measure the electrical impedance of single biological particles at high speed. The detailed information of single cells such as cell size, membrane capacitance, and cytoplasm conductivity also can be obtained by impedance measurement over a wide frequency range. In this work, we developed an integrated microneedle microfluidic device to detect and discriminate 9- and 16-μm microbeads. Two microneedles were utilized as measuring electrodes at the half height of the microfluidic device to perform measurement of electrical impedance under a presence of cells at the sensing area. Furthermore, this device was able to distinguish the cell concentration in the suspension fluid. The reusable microneedles were easy to be inserted and withdrawn from the disposable microfluidic. The ultrasonic cleaning machine has been used to clean the reusable microneedle with a simple cleaning process. Despite of the low-cost device, its capability to detect single particles at the sensing area was preserved. Therefore, this device is suitable for cost-efficient medical and food safety screening and testing process in developing countries.

Keywords: impedance, flow cytometry, microfluidics, microneedle, single cell

1. Introduction

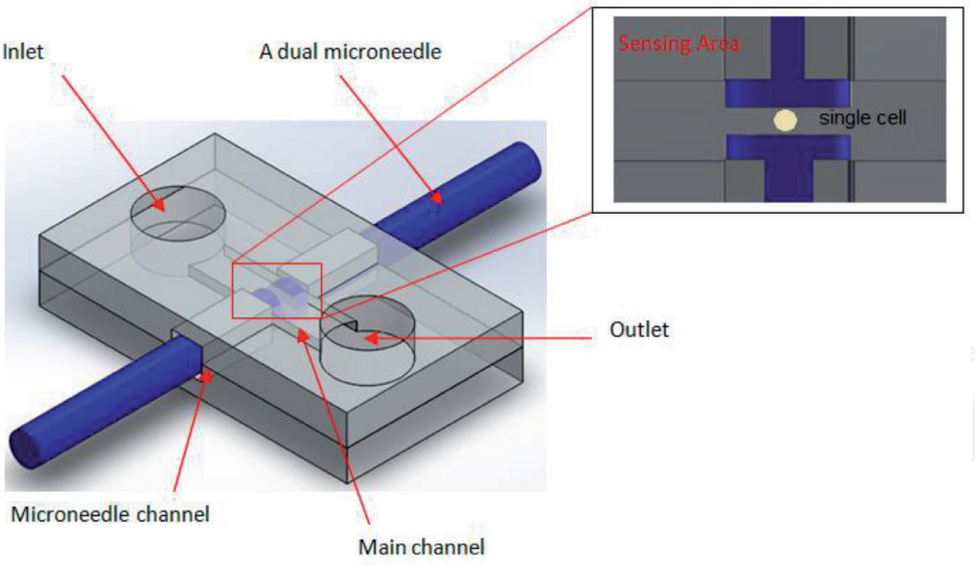
The single cell analysis (SCA) has been emphasized to provide biologists and scientists to peer into the molecular machinery of individual cells. For the application of medical diagnosis, detection of cancer cells and pathogenic bacteria cells in blood is utilized as a diagnosing infectious disease. It is reported that detection of circulating tumor cells (CTCs) in the blood has shown to be clinically important for early stage metastasis or recurrence of cancer. The presence of rare CTCs in blood is ranging from only 1–100 CTCs/ml blood [1]. *Plasmodium falciparum* malaria, which kills mainly children in developing countries infected the blood sample of patients at concentration of ~1/50 μl of blood [2]. Nowadays, the analysis of single cell in biological measurements and medical research has emerged as a distinct new field and acknowledged to be one of the fundamental building blocks of life [3]. Amongst of various single cell analysis, cell impedance measurement has become an effective method of biological measurement [4]. The physiological behavior of the cells and their corresponding molecular expressions have significant effect on the cell membrane and cytoplasm conductivity and dielectric constant, which in turn affects the overall impedance characteristics [5]. For that reason, the impedance

measurements on single cells can provide relevant information about its functional status and may be a simple and significantly less complex alternative to detailed molecular expression studies.

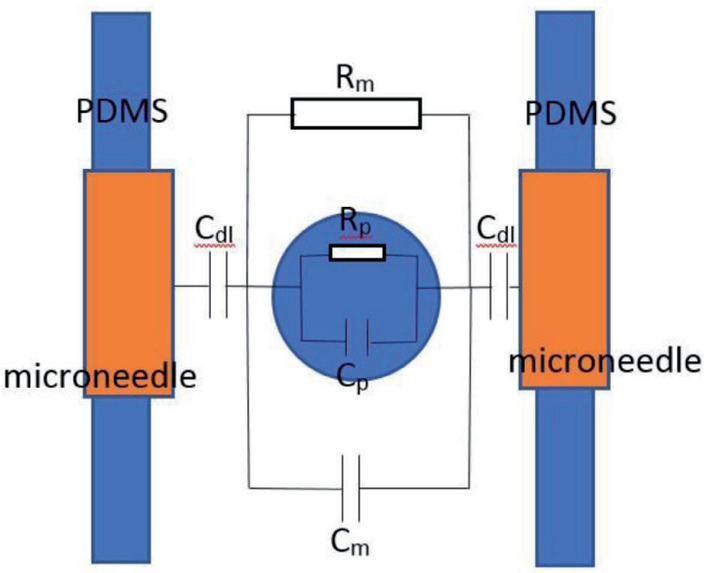
The classical method for cell detection in suspension is using flow cytometry, which is rapid and highly accurate measurement technique. Impedance flow cytometry is an indirect signal extraction from the single cells on microchannel sensing area without having direct access into intracellular region of the cells [6]. These techniques were first reported by Coulter [7] has emerged in the microfabrication device in order to analyze microscale particle with high sensitivity. However, flow cytometry involves expensive manufacturing and labelling of the cells with fluorescent antibodies [8]. Recently, the impedance flow cytometry (IFC) has gained attention for the significant promising techniques to replace and overcome the limitations associated with flow cytometry. The IFC is preferable because of fast, real-time, and non-invasive methods for biological detection. This technique is capable to be utilized as cell counting [8], cancer cell detection [9] and bacteria detection [10]. Some groups have demonstrated detection and counting of cells by using a microfluidic integrated with electrode for various electrical measurement methods in an application of food safety [11] and real-time monitoring bio-threat [12]. This measurement technique is based on the alteration of impedance across a measurement electrode due to the blocking of ionic current passing between electrodes when a presence of the cells.

The IFC is capable to distinguish and count lymphocytes, monocytes and neutrophils in human whole blood [8]. Other studies reported that IFC can detect the presence of cells based on probing the impedance inside the cell at frequency greater than 1 MHz [13]. Fabricated nanoneedles probe inside microfluidic was utilized for measuring the presence of cells at sensor surface and making it sensitive to the dielectric properties of solution [14]. However, this device requires patterning of electrode or probe on the substrate resulting in higher cost of the fabrication process. Another limitation also needs to consider is time-consuming cleaning process of the device. Several groups have demonstrated the technique to reduce the cost of microfabrication of electrodes by using printed circuit board (PCB) as a measurement electrode. They demonstrated contactless conductivity detection in capillary electrophoresis manners [15] and cell manipulation using dielectophoresis [16]. Recently, the contactless impedance cytometry was developed to reduce the fabrication cost of impedance cytometry device [17, 18]. The electrode was fabricated on the PCB substrate (reusable component) and the thin bare dielectric substrate bonded to a PDMS microchannel (disposable component) was placed onto PCB substrate. The sensitivity of this device is the limitation since the electric field was buried in dielectric substrate and not reaches the electrolyte. Several designs and method in IFC in order to detect and analyze a cell have been reported [19, 20].

This chapter discusses a novel integrated microneedles-microfluidic system for detecting yeast cell concentration in suspension as well as detecting a single particle based on the impedance measurement. The development of the device focuses on reducing the fabrication cost while preserving the main functionality, that is, cell detection. The significant fabrication cost reduction in this work is by replacing the microfabrication of electrodes by the microneedles. This device utilized a Tungsten microneedle as a measurement electrode which can be reused and easily to be cleaned. The two microneedles were placed at half height disposable microchannel to detect and enable impedance measurement of passing cells through the applied electric field. **Figure 1(a)** illustrated the schematic diagram of the proposed microfluidic chip which consists of two microneedles integrated at both sides of the microchannel. The main sensing area microchannel length, width and thickness are



(a)



(b)

Figure 1.
(a) 3D schematic diagram of the microfluidic device structure integrated with microneedle and top view of sensing area which the impedance measurement of single particle be measured. (b) Microfluidic sensing area equivalent circuit model.

100, 25 and 25 μm respectively. The device is suitable for early cancer cell detection application in developing countries since it significantly reduces the fabrication cost, that is, not required the fabrication of micro electrode.

1.1 Principle

Ohm's Law has been use as the basic principle of detecting suspended biological cells in the media. The passing cells across the sensing area have been measure by an AC current with a frequency sweep to determine the changing impedance value of media. **Figure 1(b)** illustrated the equivalent circuit model for obtain the all parameter involved in order to characterize the electrical properties of cell between two microneedles in the suspension media. The sensing area of mirofluidic chip can be modeled electrically as cell impedance of resistance R_p and cell capacitance C_p in parallel with the impedance contributed by all materials between the two

electrodes, which consist solution resistor R_m in parallel with solution capacitance C_m. Both impedance in series with a pair of electrodes capacitance double layer C_{dl}. Z_T is overall impedance of the measurement system given [21].

$$Z_T = \frac{2}{j\omega C_{dl}} + \frac{R_m R_p}{R_m + R_p + j\omega R_m R_p (C_m + C_p)} \quad (1)$$

where ω is the angular frequency of the electrical signal. As a result, the Z_T is changing according the present of cell in the sensing area. The impedance between electrode (microneedle) and electrolyte (solution medium) is our main focus in this work.

1.2 Experimental works

1.2.1 Cell culture

In this work, Sacharomycesceresiae cells and microparticle are used as a model for proof of concepts. Sacharomycesceresiae were cultivated in a petri dish containing 10 ml of YPD broth (Yeast extract Peptone Dextrose). The YPD broth contained 1% yeast extract, 2% peptone and 2% glucose. The YPD dishes were incubated at 37°C for 24 hours. The cells were washed with deioinsed (DI) water three times by centrifugation, then they were suspended in sterilized deioinsed water at various dilutions (1:10) concentration. The cells were incubated on agar plates at 37°C for 24 hours for determining the number of cells. The diameter of yeast cells varies from 4 to 7 μm. The number of cells was 1.3×10^9 colony forming units per milliliter (cfu/ml). The conductivity of DI water is 6 mS/m. The non-fluorescent polystyrene (PS) microbeads with diameter 15 and 9 μm (Polysciences. Inc.) suspended in Phosphate-buffered saline (PBS) solution were diluted to a final concentration of 1000 beads per ml. Polystyrene beads have a known size and electrical properties [22] and have constant impedance across the frequency range used in these experiments.

1.2.2 Device fabrication

The photolithography technique was utilized to fabricate the microfluidic device. The fabrication begins by designing the masks using layout editor software. The laser lithography system (μPG501, Heidelberg Instruments, Germany) has been used to write the two masks (top and bottom) on the chromium (Cr) masks. Two-step photolithography using SU-82025 negative photoresist (MicroChem, USA) was utilized to fabricate the top layer mold. The first layer has a thickness of 25 μm and was spin coated onto a silicon substrate. After pre-baking, the top layer of Cr mask was place onto the first layer of photoresist for pattern transfer by using a mask aligner (SussMicroTech MA-6), then post-baking with development. Next, the second layer with 60 μm thickness was spin coated on the first photoresist layer and pre-baking. Then, the second layer of photoresist substrate was exposed with the bottom layer Cr mask by UV light. After exposed, the top mold master was obtaining by post-bake and developed process. Meanwhile the bottom mold master with 60 μm thickness was fabricated by following the SU-8 microchannel photolithography technique. PDMS pre-polymers (SYLGARD184A) was thoroughly mixing with curing agents (SYLGARD 184B) in a ratio of 10:1 by weigh for fabricate the PDMS microfluidic chip. The mixing PDMS was poured on an SU-8 mold master (top and bottom mold master) and left for whole night cured at room temperature to obtain the PDMS microfluidic chip. To increase the bonding strength between the top side and bottom side of PDMS microfluidic chip, they were cleaned with Isopropyl alcohol (IPA) and treated by Oxygen plasma (Plasma Etch PE-25) for 25 seconds [23]. The bonding

process of both side PDMS microfluidic chip was completed in less than 2 minutes to prevent loss of Oxygen plasma effectiveness. Finally, the right and left sides of the microchannel chip were cut and leaving a square (60 μm × 120 μm) hole for inserting a microneedle. For measuring electrode, two Tungsten microneedle (Signatone) coated by parylene with tip diameter, shank diameter and length of tungsten needle are 25, 250 and 31.7 mm, respectively, was utilized.

1.2.3 Device operation

The microscope (Olympus Inverted Microscopes IX71) was utilized to monitor the sensing area of microfluidic chip system. The micromanipulator (EB-700, Everbeing) was utilized to insert the two microneedles into microchannel chip through the square hole at right and left side of the chip. For this experiment, the gap between microneedles was fixed at 20 μm. **Figure 2** illustrated the experimental setup of impedance measurement. The two microneedles were connected to impedance analyzer (Hioki IM3570) for input measuring and the result was displayed on the computer. Then, by controlling the syringe pumps (KDS LEGATO 111, KD Scientific, and USA), the 3-ml syringes of the sample solution and yeast concentration was introduced into microfluidic chip via two tygon flexible tubes.

1.2.4 Impedance measurement procedure

Standard short and open self-calibration procedure has been used for impedance analyzer in order to perform the impedance measurement. Furthermore, to calibrate the chip, impedance of 1xPBS solution was measured at the 20 μm of electrode gap. Three microfluidic devices were utilized for reproducibility testing and the experiments were conducted at room temperature. To validate the equivalent circuit model, impedance of the medium between microneedle was measured. The solutions were sterilized DI water and PBS (Photosphate-buffered saline) with conductivities 6 and 1.4 S/m respectively.

Initially, 1 ml of PBS with concentrations of 1500 mOsm was prepared for the chip cleaning process. The sample was loaded into a syringe and driven through the microchannel using a syringe pump with the flow rate of syringe pump was kept constant (60 μl/min). After flushing with PBS solution, yeast cell of 1 ml of each seven different concentrations of sample from 10^2 to 10^9cfu/ml were driven

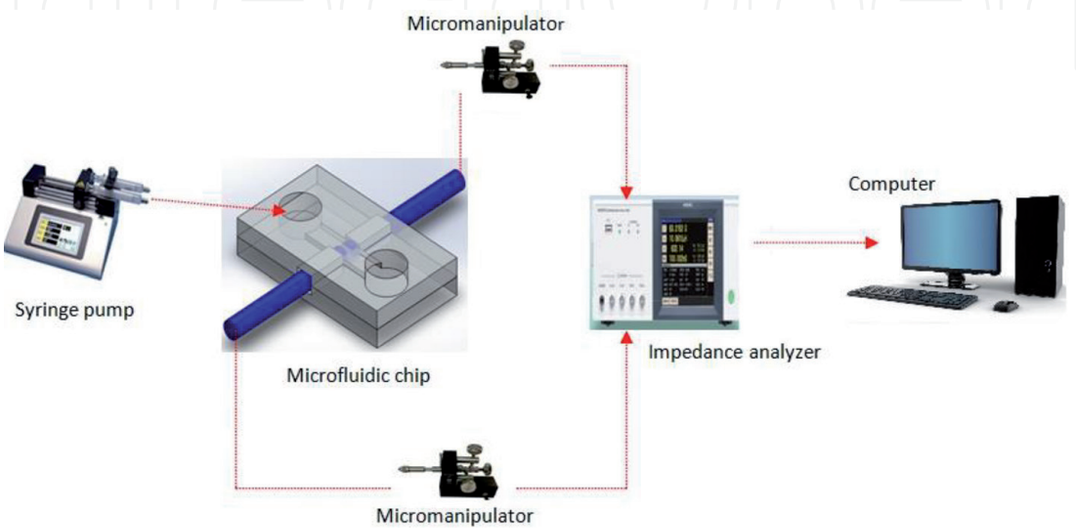


Figure 2.
The experimental set-up diagram.

through the microchannel at a flow rate of 6 μl/min. The impedance of each solution was measure by connecting the microneedles with impedance analyzer. Then, an AC signal frequency range from 100 Hz to 5 MHz with an applied voltage of 1 V was apply to determine the impedance spectra (impedance and phase vs. frequency) in order to differentiate the variations of solution samples. Between each sample measurement, the microchannel chip was flushed by DI and PBS water for 1 and 2 minutes respectively. The impedance analyzer (Hioki IM3570) GUI and post-processed in MATLAB (MathWorksInc, USA) was utilized to record the data. The impedance change during the passage yeast cells at sensing area was measured. In order to monitor the behavior of impedance for each sample, the impedance value at three frequencies (100 kHz, 500 kHz and 1 MHz) was measured.

Single cell detection and measurement was conducted based on impedance measurement with or without single cell at the sensing area. Two sample of microbeads with diameter 15 and 9 μm suspended in 1 ml of PBS with concentration of 10^3 per ml were utilized to perform this measurement and detection. Each sample were driven through the microchannel at a flow rate of 6 μl/min and measured using an AC signal frequency range from 100 Hz to 5 MHz.

1.3 Result and discussion

As a proof of concepts, the dependencies of the impedance on the various concentrations of yeast cells and a single microbead in the suspension medium by using this microfluidic device are studied. **Figure 3** presents the measured impedance spectra and fitting spectra (on a log scale) of the system for two of microchannel filled with sterilized DI water and PBS at frequency range 1 kHz to 1 MHz. For simulation, 100 data points on the impedance measured spectrum were used as input to the equivalent circuit [see **Figure 1(b)**] and generating the fitting impedance spectrum by using MATLAB. For high conductivity fluid (PBS), the result shows two domains which were an electrical double layer (EDL) region and a resistive region [24]. The EDL occurred in the low frequency range from 1 kHz to approximately 300 kHz, whereas the resistive region occurred in high frequency from 300 kHz to 1 MHz. The agreement between the measured and fitting spectra result indicated that our developed circuit model for this system is feasible to determine the impedance characteristics of solution medium.

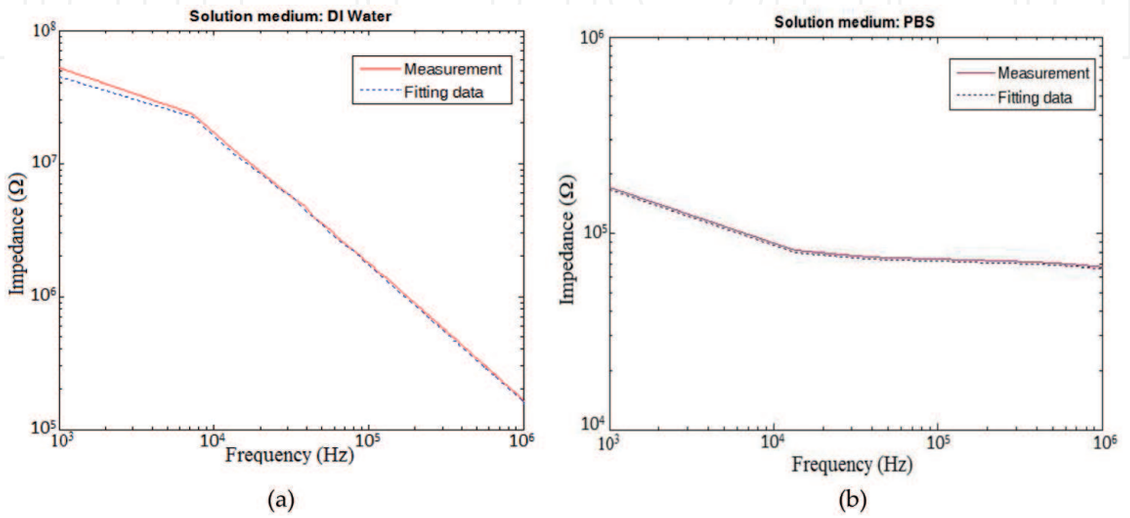


Figure 3.
Impedance spectra of sample solution together with their fitting spectra: (a) DI water (b) PBS.

To illustrate the cell detection capability of the device, yeast cell and microbeads with different concentration was utilized. Yeast cells concentration ranging from 10^2 to 10^9 cfu/ml were infused inside microchannel with fixed flow rate 6 μl/min and fixed electrode gap (25 μm). A sweep frequency (100 kHz to 5 MHz) AC signal (1 V) was applied to the one side of the microneedle and the current entering at another side of microneedle was measured to calculate the impedance of concentration of yeast cells in DI water. Initially, 10^9 cfu/ml was injected resulting in a drop-in impedance by referring the impedance of DI water as a control. Afterward, the microchannel chip was washed by the PBS followed by DI water at maximum flow rate.

The maximum flow rate of the liquid can flow inside microchannel without leaking is 300 μl/min. **Figure 4(a)** shows the impedance spectra of yeast cell in DI water with the different cell concentration in the range 10^4–10^9cfu/ml, along with DI water as a reference. After washing the microchannel, 10^8cfu/ml was infused to the microchannel resulting in an increase in impedance. It can be seen the impedance spectra of yeast cell in DI water across the sensing area (two microneedles)

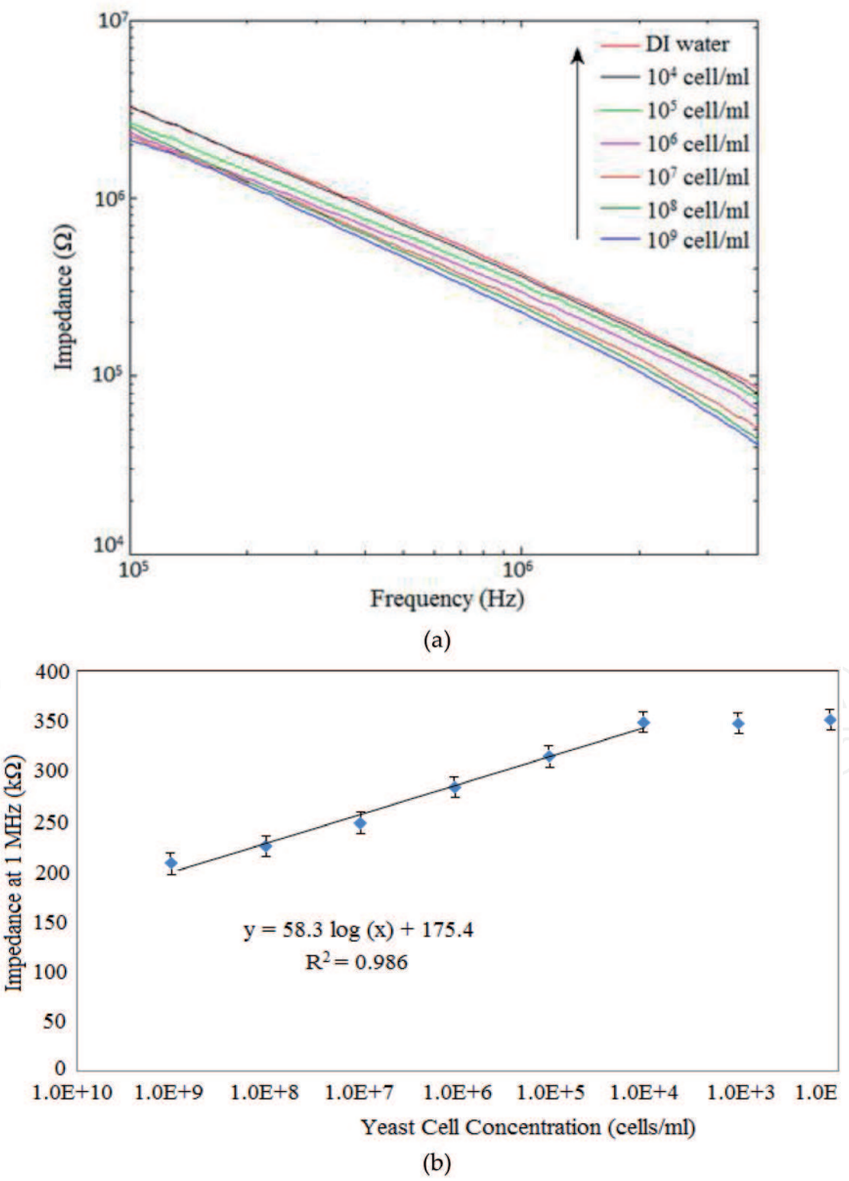


Figure 4.
(a) Impedance spectra of yeast cells in water with cell concentrations ranging from 10^2 to 10^9cfu/ml, along with DI water as controls; (b) the logarithmic value of the concentration of yeast cells and the impedance measured at 1 MHz with linear relationship.

increase with decreasing the cell concentration of cells [14]. According to the observation result, it can be said that cell suspension with high concentrations is more conductive than those with lower concentrations.

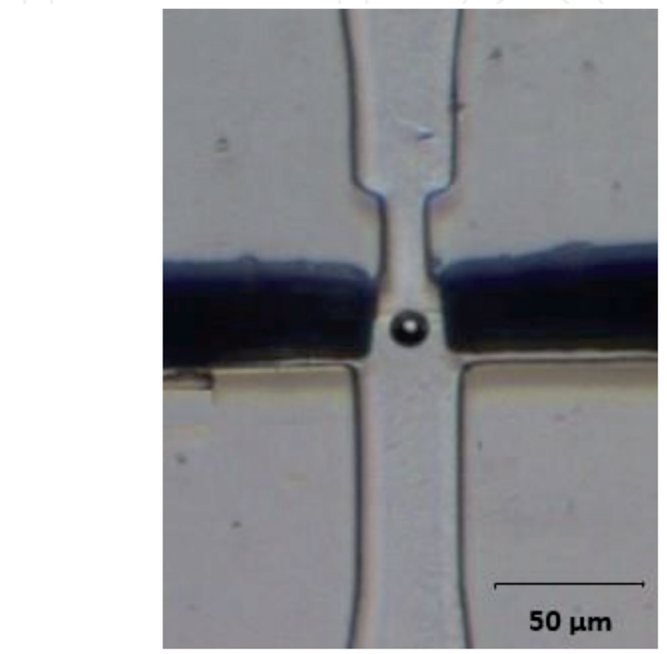
The conductivity of solution varies proportionally to the number of cell concentration at fixed volume of solution [25]. In some studies, the relative dielectric permittivity and charged polyelectrolytes inside the cell also may affect the impedance of solution [14]. The optimum region for sensing microneedle to differentiate the cell concentration in DI water occurs between 500 kHz to 5 MHz. The impedance values of the suspensions in this frequency region were significantly different from each other. The experiment was repeated two times measurement cycle and showed the similar result.

In cell detection experiment, frequency lower than 100 kHz are not considered, as the EDL started to influence the measurement at frequency below 300 kHz [17, 26]. In order to investigate the relationship between impedance value and cell concentration, we selected 1 MHz as the best representative frequency. **Figure 4(b)** illustrates the impedance responses of the sample containing different yeast cell concentrations and DI water at frequency measurement 1 MHz. The impedance of the solution was significantly increased from 207.63 to 225.42, 247.61, 284.48, 314.64, and 348.51 kΩ when the yeast concentration decreasing from 10^9 to 10^8, 10^7, 10, 10 and 10^4 cfu/ml respectively. After the cell concentrations were lower than 10^4 cfu/ml, impedance value shows no significant changing between each other or DI water. In additions, the pattern of the result shows a linear relationship between the impedance and the logarithmic value of the cell concentration at cell concentration from 10^4 to 10^9 cfu/ml (see **Figure 4**). The linear regression equation of this result is Z (kΩ) = 58.3 log X (cells/ml) + 175.4 with R2 = 0.986. The detection limit was calculated to be 1.2×10^4 cfu/ml. Error bars are standard deviations of five measurements cycle.

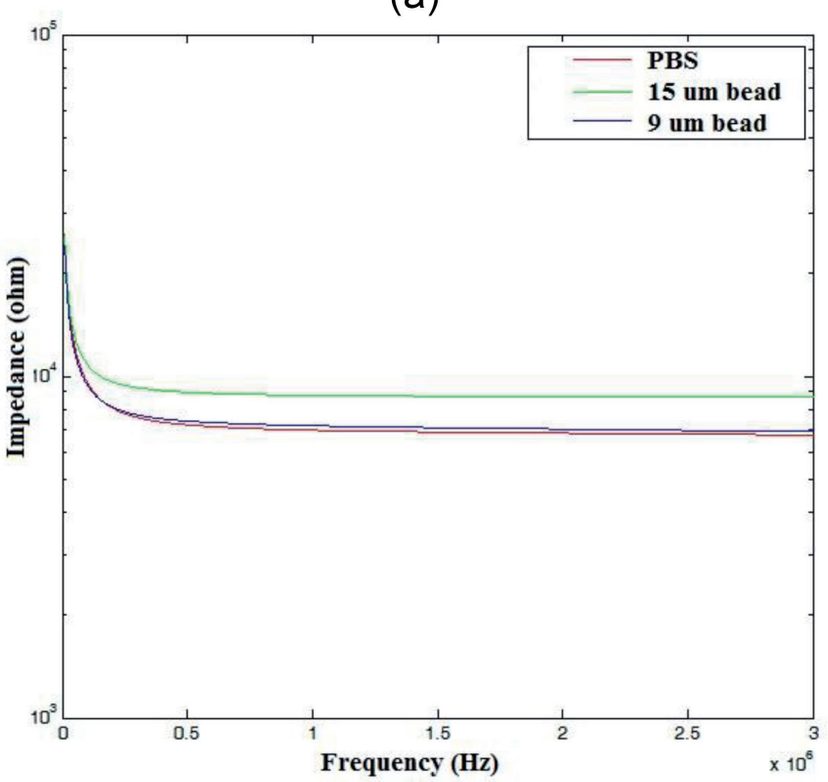
In order to measure the cell concentration in DI water suspensions, the linear regression equation of the impedance of the yeast suspensions was used. This device can be utilized to quantify cells in suspensions other than impedance microbiology and impedance biosensors for bacteria detection since the detection limit of this method is comparable with another sensor. The reported sensor for detection of pathogenic bacteria are QCM immunosensors for detection of Salmonella with detection limits of 9.9×10^5 cfu/ml [27], surface plasmon resonance (SPR) sensor for detection of *E. coli* O157:H7 with a detection limit of 10^7 cfu/ml [28] and SPR immounosensors for detection of Salmonella enteritidis and Listeria monocytogens with detection limits of 10^6 cfu/ml [29].

In order to demonstrate the capability of this device in detecting the present of single cell, two size of micro bead have been flowing inside the microfluidic device. The impedance of PBS solution as a control was initially infused inside the microfluidic device. Then two samples of microbeads in PBS solution were infused inside the microchannel with the same flow rate and electrode gap of yeast cell concentration measurement. **Figure 5(a)** shows the 15-μm microbead flow through the sensing area and a sweep frequency ranging from 100 kHz to 3 MHz AC signal (1 V) was applied to the electrode. As the result, the impedance spectrum is plotted over the field frequency, as shown in **Figure 5(b)**. The figure shows the average electrical impedance data for two size of beads and PBS solution without present of beads. From this average data, it is expected that the electrical impedance spectrum can be used to differentiate between the sizes of beads. The beads (9 and 15 μm in diameter) are clearly discriminated by impedance spectrum. The impedance increases with the increasing of the size of particle. Due to the presence of a single bead that can be regarded as an insulating object, the electrical resistance of the sensing channel was slightly increased.

As the result, we conclude this device can detect the cell concentrations in solution medium and the single microbead at the high frequency range between 100 kHz and 5 MHz. In this experiment, we did not determine the detection capability at the frequency lower than 100 kHz. For the future work, we will focus on the measurement and detection to the human cell (normal and cancer cell) the size of microneedle, single cell detection and utilize a non-polarizable electrode, that is, Ag/AgCl (to eliminate the EDL), in order to improve the performance of the device.



(a)



(b)

Figure 5.
(a) The single microbead with diameter of 15 µm flows through the sensing area. (b) Impedance spectrum of two different sizes of beads in PBS solution and PBS solution (without bead).

2. Conclusions

For conclusion, a simple, low-cost and label-free microfluidic device has developed to detect the cell concentration and single cell in the suspension medium. This device contains a disposable PDMS microchannel which allow a reusable microneedle insert into microchannel. The result demonstrated the increase of cell concentration in the solution medium were decrease the impedance value. The capability of this device to differentiate the concentration of cell from 10^9 to 10^4 cfu/ml shows the core functionality of the proposed sensor even though the manufacturing cost was significantly lower. In addition, the microfluidic device capable to detect single cell and decimate the size of single cell. As a proof of concept, yeast cell and microbeads were used in this study and we emphasize this sensing technique can be applied to a variety of cell types with diameter size in a range from 5 to 25 μm. It is recommended to perform only one measurement time for each PDMS microchip in order to avoid the potential spread of contamination to samples. The fabrication cost of this device is significantly reduces (≈ 30% fabrication cost was reduced based on facility rental and raw material usage) which is suitable for early cancer cell detection and water contamination application in developing countries.

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