Jurnal Teknologi

Full paper

Electrodes Microfluidics System for Microbio Object Analysis

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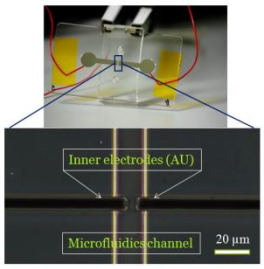
Article history

Received :5 February 2014
Received in revised form :
7 April 2014
Accepted :20 May 2014

Graphical abstract



Abstract

We have designed and fabricated an electrode microfluidics system for microbio object analysis. Two parallel-plate electrodes were fabricated using the soft lithography technique integrated with a polydimethylsiloxane (PDMS) microfluidics channel. Gold (Au) material was decomposed to fabricate the electrodes. The voltage response through the charging and discharging of the electrodes was observed using an oscilloscope. For a constant dc voltage of 5 V we obtained a time constant for the electrodes of 3.6 ms. On the other hand, it requires 850 ms to discharge completely without an external load. The capacitance of the electrodes in air (room environment) is 0.39 pF. Due to the higher dielectric constant of distilled water (80.1), in the water medium the electrode capacitance is 0.77 pF. We also measured the capacitance of the electrodes by changing the medium to microbio objects such as yeast cells (5 pF) and live bacteria cells (30 pF). The results showed that bacteria have a higher electrical capacitance than yeast.

Keywords: Parallel-plate electrodes; microfluidics system; capacitance of water medium; dielectric properties microbio object

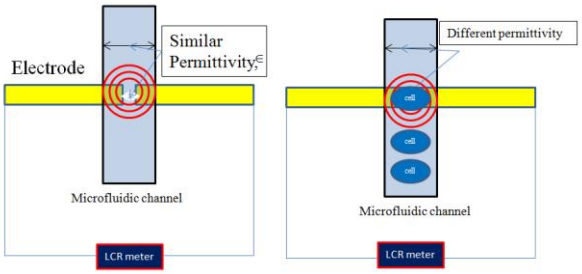
1.0 INTRODUCTION

Electrical properties, like conductivity, dielectricity and permittivity, of microbio objects such as cells, tissues and *C. elegances*, are of great interest to researchers. They are the fundamental properties of living elements [1]. These properties elucidate the complex electrical behaviour of microbio objects [2]. For instance, single cell viability was determined from the cell's electric conductive property using a dual nanoprobe [3], dielectrophoresis of the cells using living cantilever arrays [5], mechanical property analysis of the cells using microfluidic electromanipulation [6], etc. Moreover, the electrical properties of microbio objects have been studied for centuries as they are related to the thickness of membranes and also the ionic nature of cytoplasm and genes [7].

Previously, our colleagues have reported a capacitance based microchip for characterizing the volume of *C. elegances* [8]. But that work was limited to the estimation of *C. elegances* volumes only. Moreover, the scale of the electrodes was in the millimetre range, while we are proposing a micrometre-range parallel-plate electrode integrated with a microfluidics channel to analyse the microbio object's dielectric properties. Figure 1 illustrates the working mechanism of the proposed device. Figure 1(a) shows the electrodes and microfluidics channel without any objects in the channel. The capacitance of the device was measured from the fundamental equation of capacitor (C) as in Equation (1).

$$C = \frac{\varepsilon A}{d} \quad (1)$$

where, $\mathcal{E}$ is the permittivity of the medium, A is the surface area of the parallel-plate electrode and d is the distance between two parallel-plate electrodes. Figure 1(b) illustrates the electrode microfluidics system when a microbio object is presented between the electrodes. Objects are allowed to pass through the electrodes through the microfluidics channel. In the presence of bio objects, such as cells, tissue and fluidic organs, the dielectric constant of the medium will change which will cause the capacitance to change as well. As a result, the dielectric properties of the object can be extracted accurately. This analysis will elucidate the electrical properties of the single cell as well the fluidic organisms of bio objects.



(a) (b)

Figure 1 Concept of electrode microfluidic system for single cell analysis. (a) Microfluidic channel without cell (b) Channel in presence of a cell

■2.0 FABRICATION PROCEDURES

2.1 Fabrication of Parallel-plate Electrodes

Fabrication of the entire chip has been divided into two different parts; fabrication of the parallel-plate electrodes and fabrication of the microfluidics channel. Fabrication was undertaken at the Micro-Nano System Engineering Laboratory, Nagoya University, Japan. Figure 2 shows the finite element model of the parallel-plate electrodes. The length of each inner electrode is 180 µm with a gap of 10 µm and the length of the outer electrode is 8 mm. The finite element model was developed with ABAQUS CAE/6.12. The lift off soft photolithography technique was used to fabricate the parallel-plate electrodes [9]. Gold (Au) ions were decomposed on the glass surface with appropriate dimensions to obtain the desired structure of the parallel electrodes. Figure 3 (a)–(f) illustrates the fabrication procedures of the parallel-plate electrodes. Initially, the glass surface was coated with photoresist AZ 5214 (AZ E. Materials, Japan) as shown in Figure 3(a). The pattern of the electrodes was obtained by exposing the photoresist under UV light (see Figure 3 (b)). AZ 300 MIF (AZ E. Materials, Japan) was used to develop the pattern on the glass surface (see Figure 3(c)). Later, Au was sputtered onto the patterned glass surface for 4 min (see Figure 3(d)). Finally, using an ultrasonic cleaner, AZ 5214 was removed (see Figure 3(e)), leaving the remaining Au electrodes on the glass surface (see Figure 3(f)). Two wires were connected with the Au electrodes by silver (Ag) pasting on the electrode edge. The outer electrode was 8 mm long, 2 mm wide and 176 nm high. The inner electrodes were 180 µm long, 10 µm wide and the gap between them of 10 µm.

2.2 Fabrication of the PDMS Microfluidics Channel

The microfluidics channel was fabricated in polydimethylsiloxane (PDMS, SILPOT 184, Dow Corning Corp.). PDMS is inexpensive, non-toxic, transparent and biocompatible [6]. Figure 4 (a)–(d) illustrates the steps in the fabrication of the microfluidics channel. The soft lithography technique was used to develop the master mould on the silicon surface, Figure 4(a). The width and height of the channel is 20 µm and 10 µm respectively. The PDMS material was then poured onto the mould surface (Figure 4(b)) and treated at room temperature for 24 h. The PDMS replica was then peeled off from the surface and an inlet and an outlet were drilled, Figure 4(c). Finally, the PDMS microfluidics channel was ready, Figure 4(d). The fabricated PDMS channel was then aligned properly on the Au electrode surface. Alignment was undertaken carefully, so that the microfluidics channel was placed exactly between the inner electrodes. Figure 5 shows the fabricated lab-on-chip, the inset shows the microfluidics channel which is exactly between the electrodes.

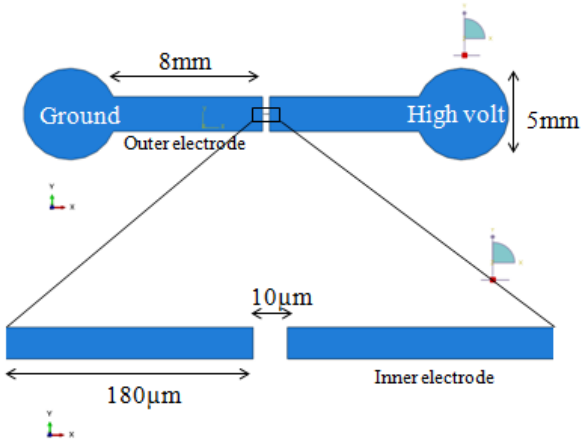


Figure 2 Finite element model of the electrode microfludics system

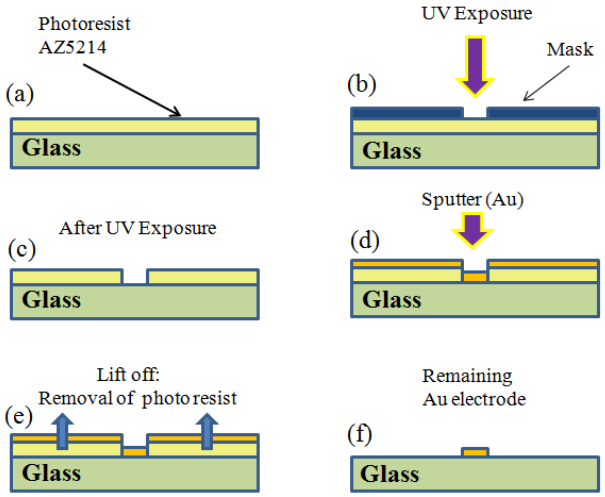


Figure 3 Lift off fabrication process of electrodes. (a) AZ5214 photoresist coating on the glass surface. (b) UV exposure on the mask. (c) Object is ready for sputtering. (d) Gold (Au) sputtering on the surface. (e) Removal of the AZ5214 photorest by ultrasonic cleaner. (f) Remaining Au electrode on the glass surface

■3.0 CHARACTERIZATIONS OF THE MICROFLUIDICS SYSTEM

3.1 Liquids Flow Through PDMS Microfluidics Channel

The PDMS microfluidics channel was developed for the flow of microliquids and micro-objects through the electrodes. We undertook several experiments to observe the function ability of the channel. Distilled water mixed with blue dye was used as the liquid sample to flow through the channel. Figure 6 shows that blue water flows through the microfluidic channel. We controlled the flow of the water using a Legato 200, Syringe micropump (KdScientific) at a 100 µl/min flow rate. The flow rate can be controlled by increasing or decreasing it using a micropump.

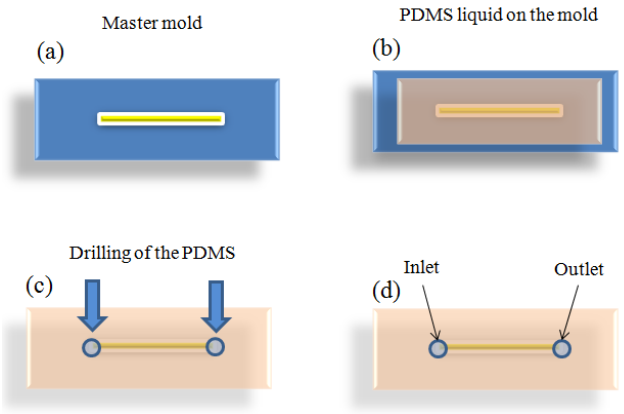


Figure 4 Fabrication procedures of the PDMS microfluidic channel. (a) Master mould after soft photolithography. (b) PDMS liquid layer on the master mould. (c) Dried PDMS structure and drilling of the channel. (d) Inlet and outlet of the microfluidics channel

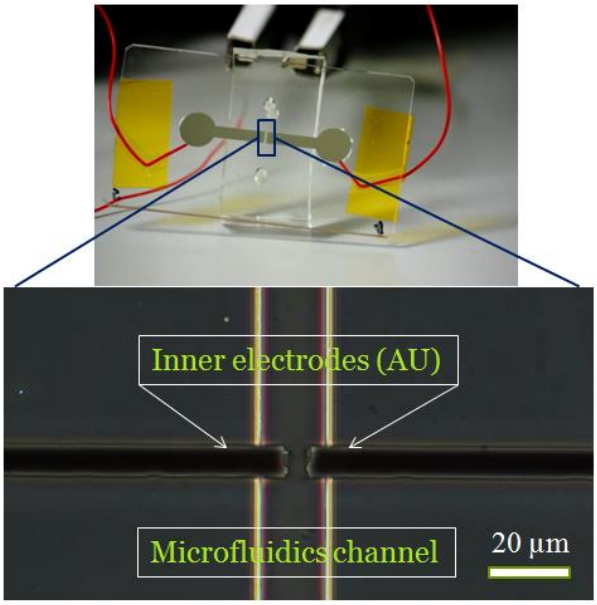


Figure 5 Fabricated electrode microfluidics system

3.2 Polystyrene Microbeads Flow Through Microfluidics Channel

Microbeads are widely used as samples of microparticles to observe the behaviour of the object inside a microfluidics channel [10]. We used polystyrene microbeads (Sperotech Inc. PP-50-10) as the sample object and the average size of the beads was 5.2 µm. Initially, the microbeads were diluted in water. The microbeads were made from polysterene and there was no significant adhesion force among the beads. As a result, upon dilution, the beads became separated and floated individually. Figure 7 shows the microbeads flowing through the microfluidics channel. From these experiments we can say that micro-objects can be infused inside the microfluidics channel and in future this device can be useful for single cell analysis.

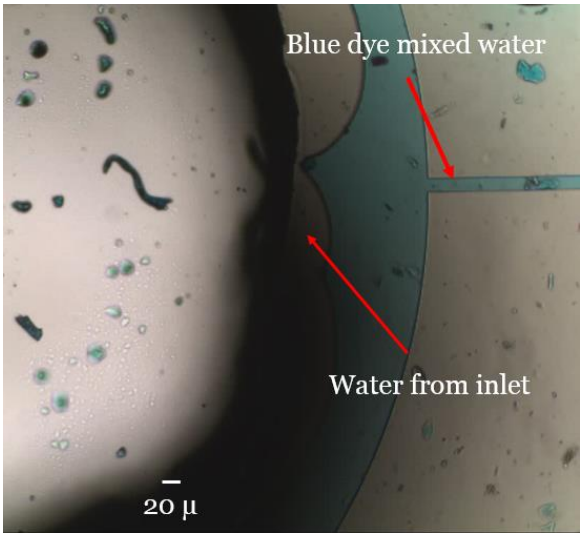


Figure 6 Water mixed with blue dye flowing through the microfluidic channel

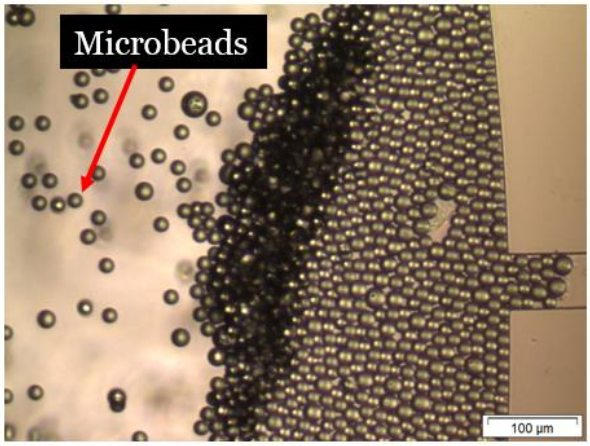


Figure 7 Microbeads inside the microfluidic channel

■4.0 VOLTAGE RESPONSE OF THE PARALLEL-PLATE ELECTRODES

Electrical characterizations of the microfluidics chip were undertaken to understand the electrical behaviour of the parallel-plate electrodes [7]. As the fabricated electrodes are parallel to each other, we expected them to behave like a parallel-plate capacitor.

4.1 Charging of the Parallel-plate Electrodes

The charging behaviour of the electrodes was analysed to extract the time constant of the parallel-plate capacitor. We applied a constant electrical potential of 5 VDC and observed the response of changing it through the electrodes. A Tektronix TDS 2014B oscilloscope was used to observe the voltage response of the electrodes. Figure 8 shows the voltage response while charging the electrodes. According to the principle of the capacitor, the time constant of this device is at 68 % of the input voltage of 3.4 V and the time constant is 3.6 ms. The time constant of the parallel-plate electrode can be adjusted using Equation (2).

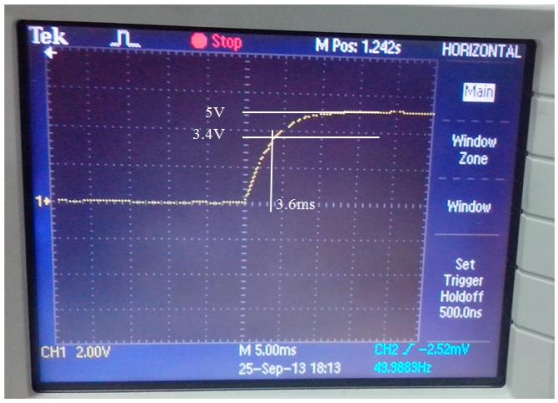


Figure 8 Charging of the parallel plate electrodes. Input voltage is 5 V. Time constant is 3.6 ms

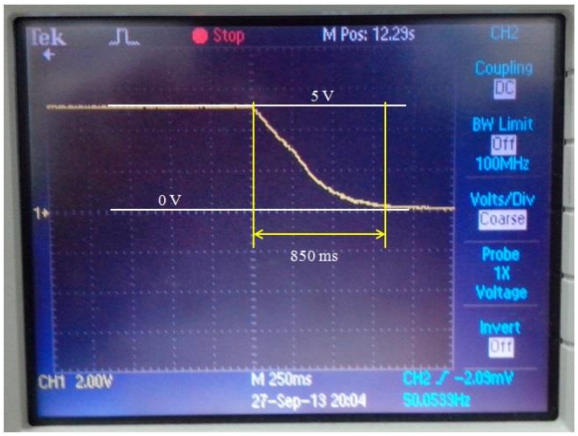


Figure 9 Discharging of the parallel plate electrodes. Time required to fully discharge is 850 ms

$$\tau = RC \quad (2)$$

where, R is the external resistor and C is the capacitance of the parallel-plate electrodes. Adjusting the time constant is important to get an accurate observation of the bio objects flowing through the microfluidics channel. However, we experienced a linear increase in voltage through the electrodes in the presence of salty water.

4.2 Discharging of the Parallel-plate Electrodes

Discharging of the electrodes was observed by switching off the power supply. We observed that the discharging time of the electrodes was 8.6 ms without any external load. The discharging time can be adjusted by adding an external resistor to the wire. The discharging time will control the sensitivity of the electrodes in terms of detecting objects. Figure 9 shows the exponential decrease in the voltage while discharging.

5.0 RESULTS AND DISCUSSIONS

5.1 Capacitance of the Parallel-plate Electrodes in Air

The capacitance of the parallel-plate electrodes was measured in a room environment with an Agilent LCR Precision Meter 4263B. Initially, the LCR meter was calibrated in a room environment. Later, we measured the capacitance of the microfluidics chip. In this stage, the medium of the parallel-plate electrodes was air and due to the air dielectric constant (1.05) the capacitance increased exponentially through the frequency. Maximum capacitance was measured as 0.39 pF. This result ensures the capacitive property of the fabricated parallel-plate electrodes.

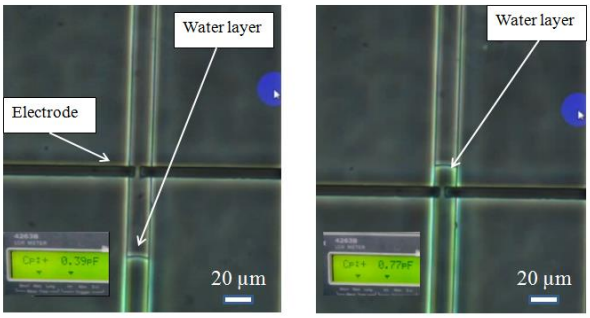


Figure 10 (a) Inset shows the capacitance electrodes without water. (b) Capacitance of the electrodes in the presence of water

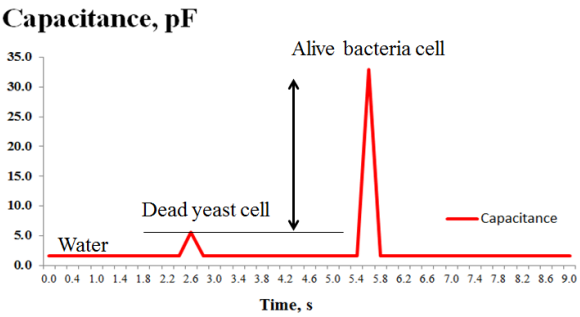


Figure 11 Capacitance of electrodes in different medium

5.2 Capacitance in Distilled Water

We also observed the capacitance of the electrodes in distilled water. We were able to flow water through the microfluidics channel. A Legato 200, Syringe micropump (KdScientific) was used to control the water flow precisely. Water flow of 5 µl/min was used to flow through the microfluidics channels. Figure 10(a) shows water pretending to cross the electrodes and the capacitance is 0.39 pF. On the other hand, the capacitance of the electrodes is 0.77 pF when water is on the electrodes (see Figure 10(b)). Due to the dielectric constant of the water (80.1) capacitance of the electrodes.

5.3 Capacitance of the Electrodes in the Microbio Object's Medium

Previously, the complicated dielectrophoresis technique has been used to extract the dielectric constant of microbio objects [11]. Our fabricated electrode microfluidics system eases this work by measuring the capacitance of the electrode in the presence of a microbio object medium. We have chosen yeast and bacteria as the samples of bio objects [12]. The yeast cell and bacteria were diluted into distilled water to flow through the channel. Figure 11 shows that the capacitance of the electrode is 5 pF for dead yeast cell while it is 33 pF for live bacteria cells. Using (1), we may calculate the permittivity of the object which

will lead us to extract the dielectric constant of the yeast and bacteria, without the dielectrophoresis technique [13].

6.0 CONCLUSION

The electrode microfluidics system has been successfully presented in this article. The results show that the fabricated microfluidics chip works like a parallel-plate capacitor by the charging and discharging of electric potential. Our proposed device is able to analyse the dielectric properties of liquids as well as microbio objects such as cells and liquid organs. The potential applications using the electrode microfluidics system will help researchers to understand the electrical properties of living elements, which may lead us to differentiate healthy and unhealthy conditions of living organs from variations in capacitance readings.

Acknowledgments

We would like to express our heartiest gratitude towards the Ministry of Higher Education Malaysia (MOHE) grant no. 78677 (FRGS), MOHE grant no. 4L038 (ERGS) and Universiti Teknologi Malaysia, grant nos. 77973 (NAS), 03H80 (GUP) and 02H34 (GUP).

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