Detection of water pathogen based on dielectrophoresis

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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

**DETECTION OF WATER PATHOGEN BASED ON
DIELECTROPHORESIS**

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SCHOOL OF MECHANICAL AND AEROSPACE ENGINEERING

2012

Detection of Water Pathogen Based on Dielectrophoresis

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Abstract

A neutral particle is polarized when it is subjected to non-uniform electric field, and upon polarization, this neutral particle will be moved by dielectrophoretic force. Neutral particles include polystyrene bead, cells, bacteria and other micro-organisms. By utilizing dielectrophoretic phenomena, separation of cells and bacteria can be achieved instantly and this separation method was proven to be effective by previous researchers. Compared to other conventional clinical separation methods, the dielectrophoresis (DEP) separation can greatly reduce the experimental cost and time.

On the other hand, water safety has been a critical global issue. More attention has been paid to the safety and cleanliness of drinkable water. Many researchers have also focused on the separation and detection of bacteria in the water medium. Although there are some existing techniques available for cell/bacteria separation currently, those techniques require highly cost and bulky equipments. Moreover, all those techniques require long processing time to separate and concentrate the samples.

The objective of this research work is to develop a dielectrophoresis-based device that is able to trap and detect the presence of live bacteria in the water medium and more importantly able to reduce the processing cost and time. The bacteria, such as *E. coli* and *E. faecalis*, which can be commonly found in polluted water medium had been selected in this research.

In this study, firstly, numerical modeling and simulations were carried out using COMSOL Multiphysics to understand better the influences of various parameters on the dielectrophoresis and on the behavior of biological particles. Subsequently, a microfluidic

chip was designed based on the dielectrophoresis phenomena and fabricated using microfabrication technique for trapping and detecting more than one type of bacteria in water. The bacteria used in this work were prepared and cultured with conventional microbiological culturing method in the environmental lab. The trapping behaviors of different bacterial species under continuous flow in the micro channel were studied and compared. Separation of different neutral particles, such as yeast cell, polystyrene bead and bacteria was achieved using the developed chip. Their separation characteristics were evaluated and analyzed. Furthermore, the effect of AC electroosmosis on the behaviors of bacteria and non-living microorganism such as polystyrene bead was investigated and compared using various different designs of electrode. Finally, electric impedance technique was proposed and employed to achieve the detection of bacteria in a real time manner. Two impedance measurement methods were used in the experiments. It was shown that the impedance technique was able to detect the changes of impedance value when there were bacteria existing in the water medium. Various conditions such as different bacterial concentration were attempted in the experiment to understand how the impedance varies corresponding to different conditions.

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CHAPTER 1

Introduction

1.1 Background

The research in biological sample manipulation and separation has been a major research topic currently. More researches have been conducted to separate or detect bio-particles, for example the separation of viable and non-viable bacteria, detection and manipulation of cancerous cells and also separation of viruses and DNA. Individual particle such as cell is a basic unit of all functional organisms i.e., its every single property behavior will greatly affect the overall performance of the body. Therefore, the successfulness in the separation and manipulation of single type of bio-particle will lead to a good stepping-stone to further research. Nowadays, numerous conventional cell separation devices with outstanding performance have been applied in many biomedical labs. However, there are a few drawbacks for such conventional devices. One of them would be the size of the equipment which is so bulky. Besides that, the operation of such devices is costly and only allows a trained person to operate. Therefore, there is a major trend to miniaturize and develop the biomedical cell separation device into a lab-on-a-chip (LOC) or micro-total analysis systems (μ TAS). Multiple functions are able to be performed in a single lab-on-a-chip system. Furthermore, compared with conventional devices, it is relatively cheap in cost and most importantly, it is easier to be operated.

Cell separation can be basically categorized into two major groups: immunological method and non-immunological method. Some fundamental biological knowledge is needed in order to separate the cell in the immunological method as this method mainly involve the binding reaction of antibody and antigen between the cell of interest and those specific antibody which has been coated with some special particles like fluorescent dye and magnetic particles. One of the famous types of immunological cell separation method is fluorescence-activated cell sorting or flow cytometry. Even though the cell separation with immunological method can yield high accuracy of separation, it requires longer period of time to achieve the final separation and the overall process is complicated. Another cell separation method which is non-immunological method is much easier to be handled and functioned. Not much biological background or biological experiment is required besides the sample preparation in this method. The difference in physical properties of the cell suspension will be the main reason in which the separation occurs. The experimental result with this method can be fast and easily obtained and dielectrophoresis, the cell trapping and detection method used in this project, is considered to be one of the non-immunological methods.

Dielectrophoresis (DEP) is defined as a phenomenon in which a neutral particle is being manipulated or separated by the non-uniform AC (Alternating Current) electric field after the particle has been polarized. Upon polarization, the particle will either move to high or low strength of electric field. When the particles move to high electric field, it is called positive dielectrophoresis, whereas if the particles move to low electric field, it is called negative dielectrophoresis. Microfabrication technique which has been utilized in the semiconductor industry has played an important role in the occurrence of the

dielectrophoresis based microfluidic. Fabrication of electrodes in the clean room in which size can go as small as few micrometers are able to produce a magnificently high electric field gradient, which is an important factor for the particle separation to occur in dielectrophoresis. The higher the electric field gradient, the higher the dielectrophoretic force will be, and thus yield an instantaneous separation result. There are a few controlling parameters which could affect the dielectrophoresis performance; for example, amplitude of the peak to peak voltage, frequency of the applied AC electric field and the conductivity of the suspending medium. It will be challenging to find an ideal combination of these parameters in order to get an optimum result. Moreover, the separations of particles are based on the difference in size and shape of the particles, as well as the conductivity and permittivity of the particles itself. Thus, it is even more difficult and challenging to achieve the separation for living cells. Since different cells would contain different amount and type of interior sub domains or ions which play an important factor in the separation based on dielectrophoresis phenomena.

1.2 Objectives and Scopes

The main objective of this project is to develop a dielectrophoresis device which is able to trap and detect the presence of live bacteria in the water medium. The types of bacteria chosen to be trapped and detected will be those bacteria commonly found from the polluted water medium. The bacteria are prepared and cultured with the conventional microbiological culturing method in the environmental lab.

The dielectrophoresis device is fabricated utilizing the microfabrication technique in the clean room. Different electrode designs are proposed and their electric field

distribution is simulated with numerical simulation. More numerical simulations, especially the simulations on the electric field distribution of the neighboring particles are done in order to have a better understanding on the dielectrophoretic behavior of the particle in the device. Different bacterial species are then tested in the device and their trapping behavior is compared and analyzed. The special characteristics of the difference in trapping behavior for different bacterial species observed in this device are then being utilized and combined with the impedance measurement technique. This enables the detection of bacteria in a real time manner and most importantly to be able to produce the information such as the bacterial concentration as well as the percentage mixture of different bacterial species in the water medium in the fastest possible way compared to the conventional microbiological method.

1.3 Outline of the Thesis

The next chapter which is Chapter 2 reviews some of the dielectrophoresis basic theory. Different separation strategies based on the dielectrophoresis phenomena are reviewed and described. Other separation methods which are categorized under both immunological method and non-immunological method are reviewed and compared. Some detection of micro organism by the means of impedance measurement with the aid of dielectrophoresis is reviewed too. Besides that, all the general methods used to count and detect the bacteria in the conventional microbiological method is reviewed in order to have a better understanding on their methods and allow the comparison with the current bacteria detection based on microfluidic technology. Finally, the dielectrophoretic model of the biological particles is reviewed at the end of this chapter.

Chapter 3 shows numerical simulation results using Comsol Multiphysics software. The main purpose for the simulation is to find out the electric field distribution and to have a better understanding of the dielectrophoretic behavior of the particle in the dielectrophoresis chip.

Chapter 4 starts to describe the fabrication process of dielectrophoresis chip and the preparation of the bacteria sample. Brief introduction on the type of bacteria used in this project and their growth process is shown in this chapter too. This is then followed by the description of the steps to culture and prepares the bacteria sample. Finally the preparation of other samples such as yeast cell and polystyrene bead as well as the bacterial staining procedures is described too.

Chapter 5 shows the detail of the experimental setup and the process of running the experiment. Equipment used in the experiment is shown and described. This chapter shows the trapping behavior of different bacterial species. Other experiments shown in this chapter include the study of different effects on the separation performance among different neutral particles and also the study of AC electroosmosis phenomena.

Chapter 6 shows the detection of bacteria with the impedance measurement method. Experimental setups for the impedance measurement are described in this chapter and all the experimental results on the impedance measurement are explained.

Chapter 7 is the conclusion of the thesis. It concludes and summarizes all the works done in this project as well as some brief discussion on the result obtained. Future work is discussed in this chapter too.

CHAPTER 2

Literature Review

2.1 Introduction

Dielectrophoresis (DEP) is a phenomenon in which a neutral particle is being manipulated or separated by the non-uniform AC electric field after the particle has been polarized [1-5]. The non-uniform AC electric field can be generated either by the specific design of electrode geometry or by incorporating some insulators in between the electrode [6-11]. The suspending medium of the particle can be either in a droplet form which stay stationary [12] or fluid flow inside a channel through the DEP chip which may require external equipment such as syringe pump to pressurize the fluid and force the fluid flow which are basically laminar flow type [13-14]. When the two voltage signals applied have constant amplitude and half a cycle phase, the particles will be levitated and move normal to the electrode plane, namely conventional DEP or cDEP. However, when the voltage signals is supplied with a quarter cycle phase sequence, the particles will be moved in a direction parallel to the electrode plane, namely traveling DEP or twDEP. Polarization occurs when the dipole moment is induced in the neutral particles caused by the non-uniform AC electric field [15]. The dipole moment occurs when equal and opposite charges (+q and -q) generate at the particle boundary.

The general resulting dielectrophoresis force generated is shown below [4, 16-17]:

$$\langle F_{DEP} \rangle = 2\pi\epsilon_m R^3 [\text{Re}\{f_{CM}\} \nabla E_{rms}^2 + \text{Im}\{f_{CM}\} (E_x^2 \nabla \phi_x + E_y^2 \nabla \phi_y + E_z^2 \nabla \phi_z)] \quad (1.1)$$

where ϵ_m is the medium permittivity, R is the radius of the sphere particle, E_{rms} is the root mean square of the applied voltage, ∇ is the gradient operator, E_i is the electric field applied on i direction with ϕ_i the phase on i direction and f_{CM} is the Clausius-Mossotti (CM) factor given by the formula below:

$$f_{CM}(w) = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*} \quad (1.2)$$

where

$$\epsilon_p^* = \epsilon_p - j \frac{\sigma_p}{w} \quad \text{and} \quad \epsilon_m^* = \epsilon_m - j \frac{\sigma_m}{w} \quad (1.3)$$

in which ϵ is the permittivity and σ is the conductivity. The subscript of p and m denote the particle and medium respectively. Since it is obvious that from equation (1.3), ϵ_p^* and ϵ_m^* are dependent on frequency, it is no doubt that the Clausius-Mossotti factor is dependent on frequency too. The real and imaginary parts of the Clausius-Mossotti factor are both dependent on the frequency too and the sign (positive or negative) of the Clausius-Mossotti factor will decide the direction of the particles heading towards [1]. The value of the frequency which causes the sign of the CM factor to changes either from

positive value to negative value or vice-versa is called crossover frequency. At this crossover frequency, no DEP force will be experienced by the particles.

Figure 2.1: The difference in interfacial polarization and the movement of particle for positive and negative dielectrophoresis.

When a particle has higher polarizability compared to the medium, more charges are produced on the inside of the particle/fluid interface and the particle will move towards the high electric field region as depicted on the left image of Figure 2.1, this is called positive dielectrophoresis. Whereas when a particle has lower polarizability compared to the medium, less charges are produced on the inside of the particle/fluid interface and the particle will move towards the low electric field region as depicted on the right image of Figure 2.1, this is called negative dielectrophoresis.

From equation (1.1), it can be seen that there are two parts on the right hand side. The first one represents the conventional DEP force whereby the electric field must be

non-uniform whereas the second one represents the traveling DEP force whereby the applied field phase must be non-uniform in order to have both DEP force applied on the particle concurrently [16]. The ∇E^2 term in the conventional DEP force also implies that the direction of DEP force is independent of the polarity of the voltage source [15]. The dielectric properties of the particles and medium play an important role in deciding whether the particles will be moved towards the strong electric field (positive DEP) or repelled from them (negative DEP) [18].

2.2 Common DEP-based Methods for Particles Separation

A number of different strategies to achieve particles separation based on the concept of dielectrophoresis have been successfully utilized by researchers in the literature. These methods have been proven to be efficient and they are described in the following section.

2.2.1 Flow Separation

This is the most commonly used method on DEP particles separation [1, 19]. A flow medium which consists of two different particles will be pumped by the syringe pump or other pressurizing mechanical equipment through a channel which consists of laminar electrodes. During the flow, the electrodes are energized, and the voltage and frequency are set to the value in which the dielectric property of those particles is differentiable. Those that undergo positive DEP will be attracted to high electric field strength (electrode edges) whereas those that undergo negative DEP will be repelled away from the electrodes and thus follow the fluid flow until the outlet of the channel and collected at a reservoir as shown in following Figure 2.2. After the particles undergo

negative DEP has been collected, the voltage is shut off and hence those particles that initially accumulated at the electrode edges will be allowed to move since no more DEP force is applied on them, and they are collected at the channel outlet in another reservoir.

Figure 2.2: Flow separation method. In diagram A, black particles were trapped on electrode (positive DEP) while white particle flow continuously with medium (negative DEP). In diagram B, voltage is turned off causing black particles to be released and follow the fluid flow [1].

2.2.2 Field-flow Fractionation

This method will normally require the type of negative DEP instead of positive DEP [1, 20-25]. With negative DEP, the particle will be levitated to a certain height and being balanced with the gravitational force. Depending on the relative densities of particle and medium, the particles will reach different equilibrium height due to their different dielectric properties. A parabolic flow will then be introduced to the channel, and thus causing those particles which are located at different height above the electrode array

plane travel in different speed parallel to the electrode plane as shown in the following Figure 2.3. Thus the total time required to reach the outlet of the channel will be different for different types of particles, and thus separation of the particles is accomplished. One major advantage of this method is that besides separation, it is also able to transport the particles as well. Furthermore, this method is able to separate more than two types of different particles as compared to flow separation method.

Figure 2.3: Field-flow fractionation method. In this diagram white particle is levitated to a higher height compare to black particle. And thus, the travelling velocity will be different for these two particles due to parabolic flow profile and hence separation can be achieved.

2.2.3 Stepped Flow Separation

This kind of separation will require longer time but its separation efficiency can achieve as high as 100% [1, 26]. Two ports are required for this method, namely inlet and outlet port. Two types of particles to be separated are located at the center of the electrode array first. One will experience positive DEP and another will experience negative DEP. When voltage source is turned on, those two different particles with different dielectric property will be located at field maxima and field minimal respectively, then a pressure flow is introduced from one side and the flow is able to push the particles which are located at field minimal to the next electrode array since the holding force of negative

DEP is weaker compared to positive DEP as shown in the following Figure 2.4. Once this is done, the voltage source is turned off, and a pressure flow in opposite direction is introduced, causing those two groups of particles to travel one step array back. The entire step is repeated until those two groups of particles reach the different port (inlet and outlet) respectively and finally collect them at two different reservoirs.

Figure 2.4: Stepped flow separation method. In diagram A, particles with different polarizability are trapped at different region. In diagram B, particles with negative DEP are pushed downstream by the flow due to weaker force experienced. In diagram C, both types of particles are pushed upstream. This process is repeated until the two different particles exit at different outlets [1, 3].

2.2.4 Travelling Wave Dielectrophoresis

The general conventional DEP are most likely able to levitate the particles above the electrode array. However, with the traveling wave DEP method, the particles are able to move in the lateral direction parallel to the electrode array as shown in the following Figure 2.5, thus no extra external pumping equipment is required to move the particles through the channel [1, 27-40]. In order to achieve the traveling wave dielectrophoresis phenomena, different signal phase need to be incorporated with equal phase offset apart. Normally the 4 signals phased with 0^0 , 90^0 , 180^0 , 270^0 are preferred. The first electrode

which the particle will pass through will have the signal 0° phase until the fourth electrodes with 270° phase, it is then followed by the fifth electrode with 0° phase again and it goes continuously for the subsequent electrodes. It is also noted that with this method, the conventional dielectrophoresis and the traveling wave dielectrophoresis can be switched alternatively by changing the frequency during the operation. One of the famous electrode configurations to produce traveling wave dielectrophoresis is the spiral electrode array. Together with field flow-fractionation method, this method is able to achieve both separation and transportation for the particles. Another interesting observation of particle movement – electrorotation, can be observed with the same concept applied by the traveling wave dielectrophoresis [30]. It is just simply a modification of the electrodes structure by placing the electrodes in the rotating manner now instead of placing them horizontally at the same plane. There will still be a connection of AC electric field phase offset by 90° for these four electrodes surrounding the particles. The reason behind the occurrence of electrorotation is due to the induced polarization would always try to align with the electric field intensity, E [30, 41]. Thus when the electric field is rotating, the polarized particle will be rotated as well.

Figure 2.5: Travelling wave dielectrophoresis method. In diagram A, particle will be move laterally when the electrode have different signal phase. In diagram B, it shows the general electrode configuration to achieve the travelling wave dielectrophoresis, namely spiral electrode array [1-2].

2.3 Other DEP-based Methods for Particles Separation

As the size of the target particles for separation and manipulation is getting smaller and smaller, the miniaturization design of the DEP chip device is getting important and critical. One of the 2D designs that are able to manipulate nanometer size particles is the floating electrode [16]. In the floating electrode design, only two electrodes are required to be connected to the external voltage source, and it definitely helps in the saving of space and thus able to meet the objective of chip device miniaturization. In this floating electrode design, the floating electrodes (electrodes which do not connect to external voltage source) can be fabricated using nanotechnology processes whereas those two electrodes that are connected to the voltage source can be fabricated using microtechnology processes. The reason on doing this is because by utilizing the nanotechnology processes, it will enable the size and the gap of the electrodes fabricated to be greatly reduced and hence increasing the DEP force that is being reduced due to the nanometer size of particle. Another possible 2D electrode design to manipulate the nanometer size particles such as DNA is to utilize the carbon nanotubes as electrodes [42]. And it has been shown from [42] that carbon nanotubes electrode has a better performance than lithographically fabricated metallic electrodes in term of trapping efficiency of the DNA.

Among other specific design of 2D planar electrode include the combined technique of conventional dielectrophoresis, traveling wave dielectrophoresis and electrorotation phenomena in just a single chip as described from [30]. Different electrodes design which are able to produce the above three phenomena are fabricated onto a single chip, thus by just varying the external voltage connection, different phenomena can be achieved for

particles separation and manipulation. With this chip design, it definitely saves the chip fabrication cost and time, and most importantly, different particle activities can occur concurrently, for example, while being levitated to certain height above the electrode arrays plane by conventional dielectrophoresis, the particles are able to move in lateral direction in parallel to the electrode arrays plane by traveling wave dielectrophoresis.

There are more and more dielectrophoresis chips that utilize the 3D design since it is proven to be much more efficient in terms of particles separation and trapping as compared to 2D microelectrodes [18, 43-46]. The 3D microelectrode basically is formed by aligning 2 identical planar electrode array on top and bottom of the channel. When compared to other 2D planar electrode design, the 3D paired microelectrode array is able to provide higher holding force and it allows a higher operating flow rate. Most importantly, the movement of the particles is more stable for the 3D design. In one of the 3D microelectrode design by [18], a dielectrophoretic gates are generated in between the top and bottom electrode array with high-frequency AC voltage. Different type of particles flowing in the medium would have their respective threshold velocity. The applications include the separation of polystyrene beads and cells (based on the combination of negative DEP and hydrodynamic force).

Moreover, there is another 3D microelectrode design in [47] which makes use of the asymmetric microelectrodes design instead of the typical symmetric and identical top and bottom electrodes array. In this 3D design, the microchannel is designed such that it is half circular at the top and flat at the bottom. The electrodes on the top surface will be in curve shape, thus the electric field generated within the medium will have a more variation in dielectrophoretic forces especially in the transverse direction (z-direction) of the flow. It is

found that from the simulation results on electric field of the asymmetric microelectrodes, the magnitude of the electric field varied along the transverse direction and there is also an induced electric field on the rear part of the microelectrodes (thus creating additional DEP force). Thus this further variation of DEP forces will be sufficient enough to even differentiate more and manipulate the particles where some typical symmetric 3D microelectrode design is unachievable. Note that the asymmetric microelectrodes type is able to further differentiate the cells in the transverse direction.

2.4 Conventional Non-DEP Methods for Particles Separation

2.4.1 Affinity-based Cell Separation (Chromatography)

Based on the property of different solubility in two different phases, which is mobile phase and stationary phase, the analytes are able to be separated [48]. The mobile phase which is normally gas or liquid carrying some components such as cells is forced pass through the stationary phase located inside a [49]. Different components inside the mobile phase will finish passing through the stationary phase in different time. The procedure for the affinity-based cell separation basically adopt the same procedure as chromatography where a flow stream of cells mixture is passed through a stationary phase which consists of antibody inside the column. When mixture of cells is passed through, the target cells to be separated is bound to the stationary phase since binding interaction occurs between the specific target cells membrane with the antibody located all along the column (stationary phase) [50]. Thus the rest of the particles will flow through the column and being collected on a reservoir first. The bound cells can then be released by changing

the buffer solution, therefore the target cells can be collected on another reservoir. This affinity-based cell separation can be grouped into immunological technique as it involves antibody reaction with specific cell membrane.

2.4.2 Fluorescence-activated Cell Sorting (Flow Cytometry)

This method is also being categorized into one of the immunological technique since it involves antibody-antigen reaction to achieve the final objective of cells separation [50-52]. This is one of the effective separation methods, but the cost involved in this method is high. Before the separation starts, the target cells are coated with specific antigen which will only bind with its respective relevant antibody where those entire antibodies are conjugated with some fluorescent dye. The mixture which consists of different cells is forced to flow through a nozzle at the bottom of the container which is vibrating in a high frequency. The reason of the vibration is to cause the formation of single droplet (which consists of single cells) from the stream of fluid inside the tubing container. However, before the droplet is formed, a laser beam is directed to stream of fluid, and at the point where the laser beam is directed, two detectors which are fluorescence detector and forward scattered light detector are aimed on the point where laser beam is shown. The fluorescence detector is responsible to detect those cells which have been attached by antibody during the initial stage of experiment and information about the inner complexity of the cells can be obtained whereas the forward scattered light detector is able to detect the scattered beam light when the cells pass through to obtain the information about the cells volume. All the information obtained by the detectors is processed by a computer to show out the result which is understandable by human. This

process is called flow cytometry. As the process continues, electrical charge is given accordingly to the fluid stream after the information is obtained and analyzed, thus different group of cell droplets will be positively, negatively or non-charged. And finally the dropping path of the droplet will change accordingly when it passes through the electrode plate, thus separation is achieved.

2.4.3 Magnetic-activated Cell Sorting

This method is categorized under one of the immunological technique too as it involves the binding action of antibody with the cell [50, 53]. Like fluorescence-activated cell sorting, antibody will bind to specific cell membrane protein before the separation takes place, however in this case the antibody is conjugated with magnetic particles instead of fluorescent dye. The mixture of the cells is then forced to pass through column which consists of magnet, thus those cells type which bind with the antibody will be retained on the column whereas those that unlabelled will pass through the column and being collected first. The magnet is then removed, and those cells which are previously retained will be flushed out by a piston and collected in another reservoir, thus separation is achieved.

2.4.4 Hydrodynamic Separation

As compared to previous method, this is a non-immunological separation technique. This separation method is simple and direct without utilizing any technically complicated equipment [50, 54]. The cells are separated based on their properties difference such as density, shape and size. Two flows are induced (one of the flow consists

of the particles or cells with different size to be separated and the other flow does not consist of any particles) and join up at the pinched segment of the microchannel. Both the flow rates are adjusted such that the particles at the pinched segment are aligned at the side wall first. As the flow of mixture continues to flow towards a broadened area, a parabolic hydrodynamic forces is induced on those two different size particles. A larger force which is towards the center of the microchannel is mainly exerted on the larger particles whereas a smaller force which is near the side wall is exerted on the smaller particles. Therefore, once the flow reaches the broadened segment, the different sized particles will be separated.

2.4.5 Aqueous Two-phase System

This method is categorized under one of the non-immunological separation technique. As the name “aqueous two-phase” implied, this method will utilize two different aqueous and mixed together to form a two-phase system within a microchannel. The kind of aqueous used is water-soluble polymers which is usually dextran and polyethylene glycol (PEG) [50]. In the microfluidic device which consists of 2 inlets and 2 outlets, dextran and PEG which are immiscible to each other are introduced from the 2 inlets respectively [55]. As the two aqueous mix up in the microchannel, a stable interface is formed throughout the microchannel, and the cells would be migrated and attached to the interface. Noting that the direction of the cell migration is perpendicular to the gravity thus gravitational force will not affect the cell migration. Prior to the attachment on the interface, the partitioning of two different types of cells is achieved mainly due to their cell surface property such as surface charge. Thus, the two partitioned cells would be collected in the 2 different outlets of the micro devices.

2.4.6 Ultrasonic Particle Manipulation

This is a non-immunological type of separation technique. With the ultrasonic standing wave applied across the fluid flow in the microchannel, different particles type will be able to be separated [50, 56]. Standing wave, also called stationary wave means the wave which remains on its constant position. The ultrasound standing wave consists of 1 pressure node (which is located at the center of the channel) and 2 pressure anti-nodes (which are located at the side wall). Pressure node means the wave with zero displacement whereas anti-node means the wave with maximum displacement. Thus particles with different properties such as size, density and compressibility will determine their flowing position along the channel (either at the center or side wall of the channel). However, it is noted that the flow inside the microchannel is laminar flow, thus once their position has been manipulated and adjusted by the acoustic force, the particles will remain and continue to flow in the same location until they reach the end of the microchannel which diverges into 3 outlets. Thus finally, the particles which are located at the center and side wall of microchannel can be separately collected in different reservoir.

2.5 Impedance-based Technique for Detection and Characterization of Bio-particles

It is no doubt that microorganism particle such as bacteria can be easily attracted or captured by dielectrophoresis force onto the DEP chip which has been shown by a lot of researchers. However most of the research results on the dielectrophoresis phenomena are only observable via microscope which means the particle movement on the DEP chip can only be observed by microscope with CCD camera. Without the microscopy tool, it is

impossible to tell how those micro particles behave under the dielectrophoresis effect.

However there is one technique that is able to realize the quantitative estimation of the micro particles such as biological cell inside an aqueous medium which is proposed in [57-63]. This technique utilizes the impedance measurement on the parallel electrodes configuration on the DEP chip. By monitoring the changes of the impedance value of the electrodes whereby results can be obtained in a real time manner, one can depict and interpret on the concentration of the particles which is being captured and attracted by the DEP chip.

For this technique, positive dielectrophoresis is preferred over negative dielectrophoresis since all the particles are needed to be collected on the electrode edges in order to maximize the impedance changes. The electrode configuration on the DEP chip is designed such that there is a gap between a few parallel rows of electrodes. This impedance measurement technique is especially feasible and practically more accurate for the measurement of biological particle such as bacteria. The reason is because the bacteria will form a pearl-chain formation once dielectrophoresis phenomena is applied to them, and this pearl-chain will fill in the gap of the electrodes and act as a bridge to connect the two parallel row of electrodes, thus the conductance will definitely be increased. The higher the concentration of the bacteria inside the medium, the faster and the longer will be the formation of the pearl-chain, therefore by monitoring the changes of the conductance or the impedance value of the electrodes over a period of time, one will be able to quantitatively estimate the concentration of the bacteria inside the medium.

Since this impedance measurement technique was proposed, more improvement has been made in order to increase its sensitivity and accuracy. One of the examples is to

incorporate the antibody-antigen reaction into the technique [64]. It has been shown that DEP force which acts upon different strain or type of bacteria does not vary much [65]. Therefore it is always difficult to separate the bacteria if the medium consists of more than one type of bacteria. Anyway, by utilizing the antibody-antigen reaction method, specific types of bacteria are able to be separated since it is known that for every bacteria cell membrane, it consists of its own protein molecules or called antigen in this case and this antigen will only combine with specific antibody. Thus immobilizing one type of antibody molecules onto the electrode gap first will ensure that the specific bacteria with antigen on its membrane will be successfully captured on the electrode gap once a dielectrophoresis effect is applied. Upon filling up the electrode gap, impedance measurement can be performed. Another example of improvement made on this impedance measurement technique is to incorporate the phenomena of electroporabilization [66-67]. This strategy is able to further increase the sensitivity of the measurement especially during the condition whereby the bacteria concentration is low. Upon capturing the bacteria on the electrode gap by positive dielectrophoresis, the electrical voltage is further increased to cause electroporabilization. During the electroporabilization, various ions inside the bacteria are released into the medium thus further increasing the conductance.

By adopting the impedance measurement technique into the dielectrophoresis phenomena, one can obtain the result instantly which is very important in the micro particle detection. The existence and the concentration of the bio-particle in a medium can be found out in a real time manner. It is always a good practice to incorporate the electrical technique into the detection of bio-particle by the means of dielectrophoresis since the electrical signal can always be sensed in the fastest way.

2.6 Conventional Microbiological Methods for Counting of Bacterial Concentration

There are several ways to count the bacterial concentration in the water medium with conventional microbiological method. And all of them rely upon the culture-based approaches. Three different methods will be discussed here and the method chosen depends on the level of concentration of the bacteria in the water. If the concentration of the bacteria is known to be very high (usually 10^7 cells/mL or above), a spectrophotometric analysis will be adopted, otherwise plate count method will be preferred since it is a much simpler method compared to spectrophotometric analysis. However if the concentration of the bacteria is known to be very low, the third method, which is membrane filtration method will be adopted.

In spectrophotometric analysis, a spectrophotometer is used to detect the transmitted light that has been passed through the water sample with bacteria. When the concentration of the bacteria is high in the water, the turbidity of the water will increase and hence the amount of the transmitted light will decrease upon passing through the water sample. The signal of transmitted light will then be converted to the reading of absorbance or optical density shown in the spectrophotometer. The absorbance value will indirectly indicate the bacteria concentration and they are directly proportional. A standard curve represents the absorbance versus bacteria concentration has to be generated for every bacterial species before any subsequent measurement on bacterial concentration can be done. However, there is one limitation for this method compared to plate count method, which is the spectrophotometric analysis will indicate the concentration of all the bacteria present in the water medium regardless of whether it is alive or dead bacteria whereas the plate count

method will only count the live bacteria and not the dead bacteria.

If the concentration of the bacteria is known not to be too high (less than 10^7 cells/mL), second method which is plate count method will be adopted. In this method, the numbers of bacteria which are allowed to grow in the nutrient medium are directly counted inside the Petri dish. The bacterial sample has to be diluted into several low concentration samples before they are transferred to the nutrient medium and incubated. Upon incubation, the bacteria will grow and form a colony forming unit (CFU) and this CFU represent a single bacterial species which has been undergone cell division and formed a colony of bacteria, therefore, it is counted as one bacterium inside the Petri dish using naked eye.

If the concentration of the bacteria is known to be very low, a third method called membrane filtration will be adopted. The sample will be forced to flow through a membrane filter consisting of grid line using a vacuum pump. The filter has a very small pore which is smaller than the size of a bacterium, and hence when the water sample with bacteria is sucked through the filter, the water will flow pass the filter leaving those bacteria to be trapped on the filter. The filter is then transferred to the nutrient medium with a sterile forceps before allowed to be incubated. CFU will be formed upon incubation, and the number of bacteria on the filter can then be counted directly under the microscope.

Besides those three methods mentioned above, there are still several ways to count the bacteria with conventional microbiological method. However, it can be seen that most of the methods rely upon the culture-based approaches which mean incubation process is unavoidable, and this incubation process usually require at least one day of time. Or in

other words, in order to detect the presence of bacteria in the water medium and to count its concentration, at least a period of one day is required which is very time consuming. Therefore, microfluidic based detection has been developed in recent years including this project to encounter this problem.

2.7 Dielectrophoretic Model of Biological Particles

Unlike any other neutral particle, biological particles have a complicated internal structure. Therefore, concentric multi-shell model have been developed in order to model the cell in this dielectrophoresis phenomena [68-71]. For example for biological with cell wall and cytoplasmic membrane, the cell can be modeled as shown in the following Figure 2.6 below:

Figure 2.6: Schematic of normal shell model, including the membrane and cell wall

The cell wall radius is r_1 . The inner and outer radius of the membrane is r_3 and r_2 . The model with permittivity ϵ_4 and conductivity σ_4 is surrounding by a membrane with permittivity ϵ_3 and conductivity σ_3 . The outer cell wall with permittivity ϵ_2 and conductivity σ_2 covers the membrane. The medium of permittivity ϵ_1 and conductivity σ_1 is surrounding the model.

The effective value of the core and the shell can be then expressed as following:

$$\epsilon_{34}^* = \epsilon_3^* \frac{\left(\frac{r_3}{r_2}\right)^3 + 2 \frac{\epsilon_4^* - \epsilon_3^*}{\epsilon_4^* + 2\epsilon_3^*}}{\left(\frac{r_3}{r_2}\right)^3 - \frac{\epsilon_4^* - \epsilon_3^*}{\epsilon_4^* + 2\epsilon_3^*}} \quad (1.4)$$

$$\epsilon_{234}^* = \epsilon_2^* \frac{\left(\frac{r_2}{r_1}\right)^3 + 2 \frac{\epsilon_{34}^* - \epsilon_2^*}{\epsilon_{34}^* + 2\epsilon_2^*}}{\left(\frac{r_2}{r_1}\right)^3 - \frac{\epsilon_{34}^* - \epsilon_2^*}{\epsilon_{34}^* + 2\epsilon_2^*}} \quad (1.5)$$

And hence now the Clausius-Mossotti (CM) factor for the cell will look as the following equation:

$$f_{CM,234} = \frac{\epsilon_{234}^* - \epsilon_1^*}{\epsilon_{234}^* + 2\epsilon_1^*} \quad (1.6)$$

2.8 Summary

One of the major advantages offered by dielectrophoresis as compared to other separation technique is that DEP is able to separate and manipulate the particles non-

invasively. This is an important characteristic especially for the manipulation of bio-particles since DEP technique avoids any direct contact on the cells which may cause any possible cell damage. Besides that, when compared to other separation methods such as fluorescence-activated cell sorting or magnetic-activated cell sorting, dielectrophoresis method would be relatively lower in cost and easier to function because those other two methods require immunolabeling to separate specific target cell and they require much more cell populations to achieve the separation objective. Furthermore it is always important to be able to detect the presence of bacteria in the water medium in the fastest possible time before the water is being supplied to the consumer. It is no doubt that the conventional microbiological method can detect and count the bacteria with high selectivity and efficiency, but it usually requires a long period of time for the detection and the processes are somehow quite tedious. However, with the impedance method adopted in the dielectrophoresis phenomena, the bacteria detection can be performed rapidly and the results can be obtained in a real time manner.

CHAPTER 3

Numerical Simulation and Study on Dielectrophoretic Particles Trapping

3.1 Introduction

In order to have a better understanding on the results obtained during the dielectrophoresis experiment, numerical simulation was carried out. In this project, powerful simulation software called COMSOL Multiphysics was used. This software serves to solve various physics and engineering applications which are commonly seen. For dielectrophoresis phenomena, electric field which is non uniform plays an important role. Therefore it is always important to figure out the electric field distribution in the DEP chip. Once the electric field distribution is known, the DEP force can be predicted because the gradient of the electric field is one of the important parameters which affect the DEP force. As COMSOL Multiphysics is able to solve multiple applications concurrently, pressure fluid flow can be introduced into the simulation too since more forces such as drag force will be experienced by the particle besides the DEP force. By incorporating all the possible forces that may be experienced by the target particles into the simulation analysis, it is possible to study and analyze the motion or behavior of the target particles in the DEP chip.

3.2 Design of the Electrode Shape

Before proceeding to do the simulation, the shape of the electrodes has to be designed and drawn. In this thesis, the electrodes were designed to be a 2-dimensional array type in the DEP chip. Figure 3.1 below shows several designs of the electrode and some of their critical dimensions.

Figure 3.1: Different 2-dimensional electrode designs and their dimensions.

The main reason to design the electrode into a 2-dimensional array type is to ensure that the total surface area of the electrode array can be large enough to be covered by all the water medium that pass through the chip and hence to efficiently trap the high amount of bacteria in the water medium during the positive dielectrophoresis phenomena. Furthermore, a 2-dimensional electrode in the chip is much easier and faster in term of fabrication when compared to a 3-dimensional where a very precise alignment is required. With this reason, the chip can be mass-produced every time for backup in case the chip is damaged or contaminated during the experiment. Noted that all the minimum dimension of the feature is 30 μm , that is because the minimum feature size of the plastic mask that can be manufactured is around 30 μm , unless a glass mask is used. This mask will be used during the photolithography process in the microfabrication of the DEP chip later.

3.3 General Electric Field Distribution

A quasi-static approximation is used to solve the potential field from the Laplace equation, $\nabla^2 V = 0$ using the COMSOL Multiphysics software. And the electric field can be found by taking the gradient of the potential, V in the DEP chip.

3.3.1 2-Dimensional Electric Field Distribution for Different Electrodes

After the drawing of electrode shape was completed in the simulation software, the next step was to do the setting for that specific designed drawing such as subdomain settings and boundary settings. The most important boundary setting in order to simulate the electric field distribution is the electrical potential. For the comparison purpose, the electrical potential for every electrode design was set to be the same which was 5 V.

Subsequently, meshing process was carried out before the electrical field distribution was solved for by the software. The following Figure 3.2 shows the final result on 2D view of the electric field distribution for all the six electrode designs (Design A – F). In this 2D view on electric field distribution, the height of the plane for all the electrode designs must set to be the same to enable comparison to be made. And the height of the plane was chosen to be $5\text{ }\mu\text{m}$ above electrode.

Figure 3.2: 2-dimensional view of electric field distribution for different electrode designs.

The main objective for this simulation is to find out the location of the highest electric field strength (field maximal) and the lowest electric field strength (field minimal) on the DEP chip for the different electrode designs. For all the six designs shown, the dark red colour represents the field maximal whereas the dark blue colour represents the field minimal. Therefore, if the particles experience positive DEP, they are expected to be trapped on those dark red colour regions whereas if the particles experience negative DEP, they are expected to be trapped on those dark blue colour regions. As shown from Figure 3.2, there is one similarity among all of these designs where all the electric field maximal appear to be located at the electrode edges, whereas the electric field minimal appear to be located on the electrode gap and especially on the electrode surface which has the lowest electric field. It was also found that their electric field strength is close to each other and in the same order which is 10^5 under the circumstances of the same voltage amplitude and similar feature size. Therefore, due to the reason that there is not much significant difference among different electrode shape, Design C was randomly chosen to be further simulated and fabricated during the microfabrication process.

3.3.2 Electric Field Distribution above the Surface of Electrodes

The fabricated DEP chip consists of many electrode arrays. However, only four electrodes were simulated here for the purpose of better visual analysis. Figure 3.3 shows the conceptual drawing of the electrode (Design C) in 3D before further simulation was done. The four electrodes are enclosed in a cavity space with a height of 25 μm which resemble the real channel height of the fabricated DEP chip.

Figure 3.3: Conceptual drawing of the electrode.

Figure 3.4: Cross-sectional view of the electrodes.

The boundary setting for the simulation was set to “zero charge” for the area surface surrounding the electrode as shown on 1 in Figure 3.4. And due to the reason that $10 V_{\text{peak-peak}}$ was assumed to be applied on the electrode in this electric field simulation, the boundary settings for the electrodes were set to “+5 V electric potential” and “–5 V electric potential” on 2 and 3 respectively as shown in Figure 3.4. Figure 3.5 below shows the graph of the electric field strength along the channel height at the electrode tip (point B) plotted by using the postprocessing mode of COMSOL.

Figure 3.5: Electric field strength at the electrode tip along the channel height.

The electric field strength along the channel height was analyzed based on the section A-A which passes through point B (electrode tip) as shown in Figure 3.5. And it shows that the electric field strength decrease drastically along the channel height. The electric field strength reduces from around 4.5×10^6 V/m at the electrode surface to around 0.2×10^6 V/m at the channel height of 25 μm . And after the 5 μm height, the electric field strength becomes relatively more constant. From this result, care has to be taken when designing the channel height of the microfluidic device since there is a direct proportionality for the DEP force and electric field strength according to the DEP theory.

And if positive DEP is utilized to trap the particle efficiently, the channel height should not be designed to be so high due to the low electric field strength there.

The following Figure 3.6 shows the electric field distribution at a constant height of $5\text{ }\mu\text{m}$ above the electrode. The main purpose is to have an overview on how the electric field changes at different location above a constant height from the electrode.

Figure 3.6: Electric field distribution at a constant height above the electrode.

According to the simulation result shown in Figure 3.6, it is again more convinced to conclude that the highest electric field strength occur at the electrode edge. The rectangular shapes along the x-axis represent the two electrodes as viewed from the side. In the graph, high electric field strength is found on those four electrode edges and it can be seen that there is total four peaks in the curve. Whereas electric field minimal is found

to appear on top of electrode surface and electrode gap. Therefore particle experiencing positive DEP will move towards the electrode edge whereas particle experiencing negative DEP will either move towards the electrode gap or electrode surface.

3.4 Dielectrophoretic Force

Once the general electric field distribution has been simulated and found, it can be substituted into the general equation of the DEP force to find the DEP force. The formulation of the DEP force can be expressed in the post processing mode of COMSOL to yield the force distribution in the device.

3.4.1 2-Dimensional Dielectrophoretic Force

One important factor which will affect the trapping capability of the particle utilizing positive DEP is the hydrodynamic drag force caused by the fluid flow. The fluid sample flow in the x-axis direction and hence will create a hydrodynamic force in the x-axis direction, therefore it is important to predict the DEP force in this direction too.

Figure 3.7: 2-dimensional dielectrophoretic force distribution in x-axis direction.

From the above Figure 3.7, it can be seen that there are both red and blue colour regions appeared on the electrode tips. However, blue colour region represents the minus sign value and it means that the DEP force is acting in the negative x-axis direction (particles which fall in this region will be attracted towards the electrode on the left) whereas the red colour region represents that the DEP force is acting in the positive x-axis direction (particles which fall in this region will be attracted towards the electrode on the right). Both the DEP force value in the blue and red colour region is symmetry with the only difference is the sign of the value. From this DEP force simulation, it can be seen that it has the same trend as the electric field strength simulation where the maximum force appeared on the electrode tips too. The following Figure 3.8 shows the DEP force with $10 V_{\text{peak-peak}}$ in the section A-A. And it shows that the maximum DEP force reaches around 4×10^{-11} N at the electrode tips (assuming a sphere particle of radius $1 \mu\text{m}$ with a real part of CM factor = 1 in the water medium).

Figure 3.8: 2-dimensional dielectrophoretic force along the section A-A at $10 V_{\text{peak-peak}}$.

3.4.2 Dielectrophoretic Force for Different Voltage Amplitude

It is important to understand the relationship between the DEP force and the voltage amplitude. So that during the experiment, one can predict how big is the increment of the DEP force when tuning the voltage amplitude. And from the general expression of the DEP force, the force is directly proportional to the power square of the electric field which is directly related to the voltage amplitude, therefore, as the voltage amplitude is increased, the DEP force will increase exponentially as shown in the following Figure 3.9.

Figure 3.9: Relationship between DEP forces for a) sphere particle and b) ellipsoid particle under different voltage amplitude at the electrode tips.

Figure 3.9 shows the maximum DEP force for two identical size but different shape particle which appear at the electrode tips under the different voltage amplitude for sphere particle (radius of 1 μm) and ellipsoid particle (length of 2 μm along major axis and radius of 0.25 μm along minor axis which is identical to the size of E. coli bacteria). It is assumed that both particles are suspended in the water medium with both having a real part of CM factor = 1. Figure 3.9 also shows that different shapes of particle (spherical and ellipsoidal) have the same relationship between DEP force and voltage amplitude. However, the only main difference is the magnitude of the DEP force (pay attention on the magnitude value on the y-axis scale for both graphs on Figure 3.9). It can be seen that the DEP force experienced by the sphere particle is much higher than the ellipsoid particle (eg. At 10 $V_{\text{peak-peak}}$, the maximum DEP force experienced by sphere particle is around 4×10^{-11} N compared to 1.6×10^{-12} N experienced by ellipsoid particle).

3.5 Hydrodynamic Drag Force

A particle will experience a force which acts against its motion in the fluid and this force is called hydrodynamic drag force. The particle in this project is not flowing by itself against the direction of fluid flow but is being transported by the fluid and trapped on the electrode array when DEP force applied. However the particle could still experience substantial drag force even though it is trapped and stay stationary since there is still relative motion between the fluid and particle as the fluid is being pumped and moved continuously. Since fluid sample is injected into the DEP chip with syringe pump, the flow rate can be thus set into the required or known value. With the flow rate set, the fluid velocity can be calculated, and this allowed the drag force to be found with the

following formula.

$$F_{drag} = 6\pi\eta r(v_f - v_p) \quad (3.1)$$

where r is the particle radius, η is the viscosity of the fluid medium and v is the velocity for fluid (with subscript f) and particle (with subscript p) respectively. In this formula, $6\pi\eta r$ is also called the friction factor for the sphere particle.

3.5.1 Hydrodynamic Drag Force for Different Channel Heights

For a particle which moves in the microchannel, a wall correction factor has to be considered and multiplied into the equation (3.1). Different direction of drag forces have different wall correction factor. However, due to the high ratio of the microchannel height to the particle radius in this project (with microchannel height equal to 25 μm and particle radius equal to 1 μm), and only the x-axis direction of the drag force is being considered, the effect of wall correction factor is negligible (since the factor is near to 1 with the ratio of 25 and it will not affect the Equation 3.1 after multiplication).

With the flow rate of 1 $\mu\text{L}/\text{min}$, the average fluid velocity is calculated to be around 0.167 mm/s. By substituting the fluid velocity into the Equation (3.1), the hydrodynamic drag force can be found. And since the drag force is dependent on the velocity, the drag force will have a similar parabolic profile as the velocity profile for the fluid flow in a microchannel as shown in following Figure 3.10. Figure 3.10 also shows the hydrodynamic drag force for different channel heights under the same 1 $\mu\text{L}/\text{min}$ flow rate and it can be seen that the larger the channel height, the smaller will be the maximum drag force produced at the center of the microchannel.

Figure 3.10: Hydrodynamic force for different channel height under the same flow rate of 1 $\mu\text{L}/\text{min}$.

3.5.2 Comparison of Drag Force for Spherical and Ellipsoidal Shape

It is known that most of the biological particles have different kind of shape rather than spherical shape, for example ellipsoidal shape. Therefore it is important to analyze if there is any difference in hydrodynamic drag force for ellipsoidal shape as compare to spherical shape because it may affect the trapping capability of the particle. Different shapes will have different friction factor. However, for ellipsoidal shape alone, it has total 3 different friction factors corresponding to 3 different particle flowing phenomena. The ellipsoidal particle can move along its lengthways direction, along its sideways direction or move randomly. And their respective hydrodynamic drag forces are expressed in the following equations.

$$F_{\text{ellipsoid,lengthways}} = \frac{8\pi\eta a_1}{2\ln\left(\frac{2a_1}{a_2}\right)-1} (v_f - v_p) \quad (3.2)$$

$$F_{\text{ellipsoid,sideways}} = \frac{16\pi\eta a_1}{2\ln\left(\frac{2a_1}{a_2}\right)-1} (v_f - v_p) \quad (3.3)$$

$$F_{\text{ellipsoid,random}} = \frac{6\pi\eta a_1}{\ln\left(\frac{2a_1}{a_2}\right)} (v_f - v_p) \quad (3.4)$$

where a_1 is the half of the major axis of the ellipsoidal particle and a_2 is the half of the minor axis of the ellipsoidal particle.

By incorporating the above equations into the COMSOL post processing mode, the drag force under different phenomena against the flow rate can be plotted as shown in following Figure 3.11.

Figure 3.11: Maximum hydrodynamic force against flow rate for different shapes of particles.

As shown in Figure 3.11, the relationship between the drag force and flow rate is linear as compared to the exponential relationship between the DEP force and voltage amplitude. The result also shows that spherical has a higher drag force compared to ellipsoidal at the same flow rate. And among the three different moving phenomena of ellipsoidal, the one moving in lengthways direction tend to experience the highest force. From this result, it also shows that under the normal operating parameters (with flow rate usually set to be around 1 $\mu\text{L}/\text{min}$, and voltage amplitude usually set to be around 10 $V_{\text{peak-peak}}$ in this project), the maximum hydrodynamic drag force which always appeared at the center of the microchannel is in the order of 10 times smaller than the DEP force on the electrode surface as compared with Figure 3.9. And with the parabolic profile, the drag force on the electrode surface will reduce to an even much smaller value (around $3.13 \times 10^{-13} \text{ N} \ll 4 \times 10^{-11} \text{ N}$ of DEP force from the calculation when assuming the drag force act on the center of the 1 μm radius particle). Or in other word, particle flowing near or on the electrode surface will be captured and trapped instantaneously and efficiently on the electrode surface due to higher DEP force under the normal experimental operating parameters.

3.6 Buoyancy and Gravitational Forces

When a particle is fully submerged inside the fluid, it will experience a force in an upward direction and it is called buoyancy force. According to Archimedes's principle, the volume of the fluid displaced by the particle is the same as the volume of the particle itself, and the buoyancy force is equal to the weight of the displaced fluid based on the following equation.

$$F_{buoy} = \rho_m V_p g \quad (3.5)$$

where ρ_m is the density of the fluid, V_p is the volume of the particle, and g is the acceleration due to gravity.

However, the particle will also experience a downward force in the fluid which is called gravitational force and it is equal to the weight of the particle.

$$F_{grav} = mg = \rho_p V_p g \quad (3.6)$$

where $m = \rho_p V_p$ is the mass of the particle, and ρ_p is the density of the particle.

The particle will float or sustain at a certain height in the fluid if the buoyancy force is equal to the gravitational force without consider any other external force. Therefore it is important to have some brief idea on how much buoyancy and gravitational force experienced by the particles especially for bacteria and yeast cell which were used in this experiment. Two important parameters for this calculation are the mass and the volume of the particle. And upon calculation using the above equations, the gravitational force experienced by the bacterium and yeast cell is in the range of 10^{-12} N and 10^{-11} N respectively. Whereas the buoyancy force experienced by the bacterium and yeast cell is in the range of 10^{-15} N and 10^{-13} N respectively. From this calculation, it is found that the buoyancy force which is acting upward is negligible when compared with the gravitational force which is acting downward for both species (buoyancy force is 100 to 1000 times smaller than gravitational force). Therefore, there will always be a sedimentation force (due to gravitational force) which pulls down the particles towards the bottom of the channel under any conditions. And the sedimentation force experienced by

the yeast cell is much larger than the bacterium due to higher gravitational force and thus the yeast cell will sink towards the bottom of the channel in a relatively faster time than the bacterium under the same condition.

3.7 Electric Field Strength Distribution of 2 Neighboring Particles

For the bacterial testing in this project, it will always involve a huge amount of particles concentration in the water medium due to the difficulty in preparing just a single bacterial cell from the conventional microbiological bacteria culturing method. Therefore, it is important to analyze the electric field distribution of two neighboring particles, and study how the electric field distribution surrounding the particles will affect their DEP performance.

In COMSOL Multiphysics, two sphere particles which either exhibit similar dielectric properties (two of them exhibit positive or negative DEP together) or different dielectric properties (one exhibit positive DEP and the other exhibit negative DEP) were included in the simulation showing the analogy of the mixture of bacterial or other species in the water medium. The electric field distribution surrounding these two particles was simulated in order to have a better understanding on their DEP behavior.

3.7.1 Similar Dielectric Particles Aligned Along with Electric Field

Figure 3.12: Electric field distribution of two neighboring particles align along with electric field.

The above Figure 3.12 shows the electric field distribution around the two particles which align along with the electric field. Red colour represents high electric field strength whereas blue colour represents low electric field strength. The image on the left shows the two sphere particles which experience the positive DEP and the one on the right shows the two sphere particles which experience the negative DEP. The simulation result shows that when a particle is more polarizable than the medium (positive DEP), its internal will exhibit low electric field which agree with the DEP theory. And it can be seen that the sides of the particles consist of high electric field strength whereas the top and the bottom of the particles consist of low electric field strength. It is known that when particle experiences positive DEP, it will be attracted towards the strong electric field region, therefore the two sphere particles will attracted to each other since there is high electric field region appear in between them. If there are more particles, they will be joined side by side along the electric field, forming the famous phenomena, known as pearl-chain liked formation.

However, for the particles which experience negative DEP, it was shown that the internal of the particles exhibits high electric field. And there is weak electric field in between the particles. On the other hand, the top and the bottom of the particles experience high electric field strength. And again, the two sphere particles will be attracted to each other too and form a pearl-chain type of formation along the electric field (if there are more particles) since it is known that when particle experiences negative DEP, it will be attracted towards the weak electric field region.

In conclusion, when there are two or more particles with similar dielectric properties regardless of whether they are experiencing positive or negative DEP, their stable orientation will be in parallel or along the direction of electric field.

3.7.2 Similar Dielectric Particles Aligned Perpendicular to Electric Field

Figure 3.13: Electric field distribution of two neighboring particles align perpendicular to electric field.

From the above Figure 3.13, it can be seen that when the two sphere particles experience positive DEP, the sides of the particles consist of high electric field strength

and the top and bottom of the particles consist of low electric field strength whereas for negative DEP, the sides of the particles consist of low electric field strength but high electric field strength on the top and bottom of the particles. And with such orientation where they are aligned perpendicular to the electric field, low electric field appears in between the two sphere particles for positive DEP and high electric field appears in between the two sphere particles for negative DEP. With this kind of electric field distribution, the two particles will not be attracted to each other in the vertical direction (since positive DEP particles attracted towards strong electric field and negative DEP particles repelled away from strong electric field) therefore this kind of orientation is unstable. The two sphere particles will eventually move and orientate in such a way where they will align parallel or along the electric field as in previous case.

3.7.3 Different Dielectric Particles in the Electric Field

Figure 3.14: Electric field distribution of two dissimilar dielectric properties in different orientation.

As compared with the two previous cases, now the two particles in the electric field possess different dielectric properties, one with positive DEP and another with negative DEP. The electric field distribution for this case is shown in Figure 3.14. The orientation for the particles which align along the electric field as shown on the left side of Figure 3.14 is unstable. That is because the particle with positive DEP (blue colour) tends to be attracted towards the top or bottom of the particle with negative DEP (red colour) since there is high electric field strength region. The same phenomenon goes for particle with negative DEP whereby it will be moved towards the top or bottom of the particles with positive DEP since there is low electric field strength region. However, for the particles which align perpendicular to the electric field will be stable since they will be attracted to each other directly in the vertical position.

In conclusion, two particles of similar dielectric properties will always tend to align along the electric field stably, whereas two particles of dissimilar dielectric properties will always tend to align perpendicular along the electric field stably.

3.7.4 Electric Field Strength for Particles with Different Permittivity

It has been discussed on the previous section that two similar particles tend to align along the electric field. However, two similar particles may still exhibit some differences in their DEP behavior. For example, even though two similar particles which both experience positive DEP will be attracted towards the electrode edge, but the magnitude of the DEP force experienced by them can be different. This is due to the reason that under the high frequency, even though the two particles may both have higher permittivity than the medium (hence both experience positive DEP), one may have

relatively higher permittivity than another. Figure 3.15 below shows the comparison on the electric field strength for particles which have higher and lower permittivity under the positive DEP.

Figure 3.15: Comparison of electric field strength for neighboring particles with different permittivity under the positive DEP. Red curve represents particle with higher permittivity. Black curve represents particle with lower permittivity.

Figure 3.15 shows the electric field strength distribution where one particle (with radius 1 μm) has been attached onto the electrode tip while another particle at distance of 2 μm away. It can be seen that the electric field strength is the highest on the electrode tip for both type of particles (the particle with lower permittivity has higher electric field strength than the particle with higher permittivity), thus particle will be attracted on the electrode tip during positive DEP. However, the electric field strength drops drastically

inside the particle during positive DEP at location “a” shown in Figure 3.15. At the further location “b”, the electric field strength increases again. From the simulation results, it can be seen that the increment of the electric field strength for the particle with higher permittivity is much larger than the particle with lower permittivity at “b”. The electric field strength then drops gently again at the gap between the two particles since the location is further away from the electrode tips. When it comes to the next particle at “c”, the electric field strength will drop again drastically in the internal of the next particle and increase back again at location “d”. It can be seen that the increment of the electric field strength is once again much larger for the particle of higher permittivity than the particle of lower permittivity at location “d”.

From this simulation results, it can be concluded that the particle of higher permittivity will experience higher DEP force at location “b” and “d” due to the higher electric field strength (as shown from the simulation results above). Due to this reason, the particle can easily attract its adjacent particles and forms a pearl-chain liked formation with a relatively longer overall pearl-chain length.

The following Figure 3.16 shows the electric field strength after the pearl-chain is formed. It can be seen that several particles have been joined and lined up to form the pearl-chain liked formation. And the electric field strength is decreasing gradually from the electrode tip until the inner surface of the last particle. However, at the outer surface of the last particle, a sudden increment of the electric field strength is observed. Once again, the increment for the particle of higher permittivity is much larger than that for the particle of lower permittivity. It can be concluded that the pearl chain of particles with higher permittivity will experience higher DEP force and hence overcome the hydrodynamic

drag force to form a longer pearl-chain.

Figure 3.16: Comparison of electric field strength at the end of the pearl-chain liked formation with different particle's permittivity under the positive DEP. Red curve represents particle with higher permittivity. Black curve represents particle with lower permittivity.

3.8 Summary

It is always important to be able to predict the electric field distribution in the DEP chip because it plays an important role in the dielectrophoresis behavior of a particle. With COMSOL Multiphysics software, the electric field distribution in the DEP chip was simulated. And with the post processing mode of COMSOL Multiphysics, the DEP force was predicted too. Other forces which may experience by the particle inside the DEP chip

such as hydrodynamic drag force and gravitational force were also studied and analyzed. And it was found that the particle flowing on or near the electrode surface can be trapped instantaneously by the DEP force under the positive DEP due to a much higher DEP force compared to hydrodynamic force in the particle flowing direction, x-axis direction. Besides that, the electric field strength distribution of two neighboring particles was simulated with COMSOL Multiphysics. This is to allow the prediction and understanding of the stable orientation of similar and dissimilar particles and the behavior of pearl-chain liked formation under the DEP phenomena.

CHAPTER 4

Microfabrication of DEP Devices and Bacterial Samples Preparation

4.1 Introduction

The dielectrophoresis chip or DEP chip in this project was fabricated by utilizing the microfabrication technique which is widely used in the fabrication of semiconductor devices. With the microfabrication technique, a small scale dimension in the micrometer range of metallic electrode arrays was fabricated on the silicon or glass wafer. Thus all the fabrication of DEP chip was to be done inside the clean room in order to prevent any contamination to the chip. The dielectrophoresis manipulation on micro particles is achieved by the non-uniform high electric field, therefore in order to create the high electric field, there should have positive and negative electrodes with high voltage applied across them. However, if the voltage amplitude is too high, it would cause damage to the micro particles, and this is especially crucial when it is applied to the living micro-organisms as high voltage will cause the living cell or bacteria to death. Therefore in order to create a high enough electric field with low voltage amplitude to prevent any cell damage, micro scale electrodes is the best solution. And to build a micro scale electrode arrays, microfabrication process is required.

The bacterial sample was prepared and cultured utilizing conventional microbiological method. The preparation of the sample was to be done in the

environmental lab with all the necessary safety measures taken in order to prevent contamination of the bacterial sample.

4.2 Microfabrication of Dielectrophoresis Chip

In this work, 4" silicon wafer was used. Both silicon and glass wafers have been used in this work. The steps of the microfabrication process are similar for both types of wafers except that thermal oxidation process is required for silicon wafers. As there are large arrays of metallic electrodes fabricated on top of the wafer, and electrical power is supplied on those micro scale electrodes, it is always an important precaution to prevent any short circuit to occur. By having an oxidation layer which is silicon dioxide (SiO_2) in between the silicon wafer surface and the metallic electrode, short circuit can be prevented because silicon dioxide is a good electric insulator. However, thermal oxidation process is not required for glass wafer since glass is considered a good electric insulator. Upon the microfabrication of the electrode arrays, microfluidic channel has to be built to allow the continuous flow of the water medium and to complete the whole fabrication of the DEP chip.

4.2.1 Microfabrication of the Electrode Arrays

The first stage of microfabrication process basically starts with the design of mask. In this project, the mask designing software called L-Edit is used. Different electrode designs are drawn with this software before a mask can be fully fabricated. Mask or sometimes called photomask is an important tool in the microfabrication process. It enables the different design of features to be created on the wafer. The various shapes of

the electrodes come from the different design patterns of masks and those patterns are transferred to the wafer through the photolithography process whereby some specific design location in the mask will either permit or block the light which is usually ultraviolet light to pass through. There are two types of masks available in the market, one is the glass type and the other is plastic type. Glass mask is more expensive due to its complexity and high precision fabrication method. However, it is able to produce micro features of much higher resolution. Plastic mask is easier to be fabricated and costs much less. However, it has limited resolution and is not easy to be cleaned after each usage. In the project here, the plastic mask was chosen since the minimum feature size of the designed electrodes in the DEP chip is within the minimum resolution of the plastic mask.

Prior to the fabrication process, the wafer has to be cleaned with piranha solution (70 % sulfuric acid, H_2SO_4 and 30 % hydrogen peroxide, H_2O_2) in order to ensure its cleanliness and remove any residue particles. Except for glass wafer, the silicon wafer will then have to undergo oxidation process to grow an oxidation layer which is silicon dioxide (SiO_2). For silicon material property behavior, it will automatically form silicon dioxide itself even under the normal condition of room temperature, but the growth rate is extremely slow. Therefore in order to accelerate the growth rate of silicon dioxide, thermal oxidation process is still the best solution. The oxidation process is achieved inside the oxidation furnace with a very high temperature applied on the silicon wafer, and the silicon wafer will either be exposed to the water vapor or oxygen molecule, which basically categorized the thermal oxidation process into two main categories, wet oxidation (water vapor) and dry oxidation (oxygen molecule). The main difference for these two types of thermal oxidation is the growth rate of silicon dioxide and its layer

quality. For wet oxidation, the growth rate is faster and thus it is able to grow a much thicker oxide layer as compared to dry oxidation, however, the quality of the oxide layer grown by wet oxidation is not so good because it may allow current to leak through. And this is totally a reverse condition for dry oxidation. Even though the grow rate of oxidation is much lower in dry oxidation, the oxide layer quality is much superior. Therefore in most of the cases in which thick silicon dioxide has to be grown, it is preferably to grow in a manner of wet oxidation process in between the beginning and ending of the dry oxidation process. This is to ensure that the bottom and top layer of the silicon dioxide layer, which contact on both the silicon wafer and metallic electrode respectively, have a good quality layer surface. Tystar oxidation furnace was used to grow 1 μm of silicon dioxide in this project for silicon wafer.

The wafers were then undergone the vapor priming of HMDS (hexamethyldisilazane) process with the machine Delta 150 VPO. The HMDS layer formed on top of the wafer surface will increase the adhesion between the wafer and the photoresist. Once this process was done, the next stage, which is the spin coating of photoresist, must be done immediately after the wafers were cooled in ambient temperature for 1 min. Photoresist is a chemical substance which is sensitive to the exposure of light. Upon exposure of UV light, the photoresist will either become soluble or insoluble depending on the type of photoresist used. There are two major types of photoresists, which are called positive photoresist and negative photoresist. For positive photoresist, those area exposed to UV light will become soluble to the chemical developer solution whereas for negative photoresist, those area exposed to UV light will be cross-linked and become insoluble to the chemical developer solution. In this project, positive

photoresist AZ9260 and AZK7220 with thickness of 3 μm and 2.5 μm were used for the silicon and glass wafer respectively with Delta 80BM Spin Coater and CEE Spin Coater.

After the coating of photoresist, the wafer went through the soft bake process. The baking process was done by just simply putting the wafer onto the hot plate to bake at 110 $^{\circ}\text{C}$ for 4 min (for silicon wafer) or 110 $^{\circ}\text{C}$ for 90 s (for glass wafer). The main reason for the soft bake process is to enhance the adhesion of the photoresist on the wafer. Besides that, the baking process will also help to reduce the shear stress created during the spin coating process and remove the excessive photoresist solvent. The wafers were then cooled down in ambient temperature for 1 min before went through the photolithography process where Karl Suss MA6 Mask Aligner was used to transfer the pattern on the mask into the surface of the photoresist. The exposure time of the ultra violet light was set according to the thickness of the photoresist, and it is normally directly proportional to the thickness as more energy (high exposure time) is required to cause chemical reaction of thicker photoresist.

After the photolithography process, the wafers were post-baked for 60 s at 110 $^{\circ}\text{C}$ (for glass wafer only) before the development process. In this developing stage, the electrode design was realized and those parts of photoresist, which was exposed to ultra violet during the photolithography stage, would be removed by a chemical solution of eluted AZ 400K developer mixed with DI water in the ratio of 1:2.5. For glass wafer, different developer was used, it was AZ 300MIF without any dilution with DI water. A fixed amount of developer was poured into a flat container and the wafer was then immersed inside the developer. While immersed in the developer, the flat container was shook gently to realize the electrode design. The wafer was then rinsed by DI water for a

few times and spins dried.

After the developing process, the wafers were cleaned with Oxygen plasma with Reactive Ion Etching Machine (Technics Micro-RIE Machine Series 800-II) for 5 min to ensure a clean surface before the sputtering process. Sputtering process is one of the physical vapor deposition methods which deposit thin film material onto the wafer substrate. Coaxial Magnetron Sputter was used in this project to deposit 150 nm thickness of gold onto the silicon wafer substrate (platinum for glass wafer). However, in order to increase the adhesion between the metal material and the wafer substrate, 25 nm thickness of chromium (titanium for glass wafer) was first deposited before the deposition of metal. The electrode on the wafer was then realized with lift-off process where the wafer was soaked with acetone in the ultrasonic bath. During the ultrasonic bath, attention must be taken to prevent the wafer from over doing the ultrasonic bath as the excessive ultrasonic energy will also stripe off the wanted metal layer, hence damage the electrode design. Finally, the wafer was rinsed with IPA or ethanol solution and finally rinsed with DI water and spin dried.

The following diagram illustrates the overview on the microfabrication of the electrode arrays.

After development, those part of photoresist that shown by UV light is removed.

Gold is deposited on top of the whole surface area by sputtering process.

Remaining photoresist including those gold layers on top of resist is removed in the lift-off process, leaving the final design of electrode.

Figure 4.1: Final layout of the wafer after microfabrication.

4.2.2 Fabrication of the Microfluidic Channel

Once the microfabrication of the microelectrode has been completed, a microfluidic channel needs to be built to allow the sample medium to be continuously flown pass the electrode. The microchannel was built by attached a 25 μm thick pressure-sensitive doubled-sided adhesive tape (ARclear™ 8154, Adhesives Research, USA) in between the PMMA (polymethyl methacrylate) and the glass wafer. This adhesive tape would act as a spacer in the microchannel. The 1 μm PMMA was cut through two 0.5 mm diameter holes for the inlet and outlet flow with counterbore of 2 mm diameter using laser cutting machine (M-300, Universal Laser Systems, USA). The purpose of this counterbore is to ensure the fitting which join the tubing to sit in nicely, in order to minimize the possibility of leakage. The adhesive tape was cut into a required shape using the same laser cutting machine, and it was aligned visually and bonded to the glass wafer to

surround the whole electrode array followed by the bonding of the PMMA forming a cavity space with microchannel length: 33 mm, microchannel width: 3 mm and microchannel height: 25 μm . Upon bonding of the PMMA to the glass wafer, silicone tubing (Cole-Parmer, Inc. Co., IL) with inner diameter 0.063" and outer diameter 0.125" was used to join on the two holes of the PMMA. The end of the tubing was press fitted with a cut fitting (Cole-Parmer, Inc. Co., IL) first before they were joined to the PMMA with an epoxy (Plastic Steel Epoxy, Devcon, USA). The schematic diagram of the microfluidic channel is shown in the following Figure 4.2 and the assembly of the DEP chip is elaborated in the Figure 4.3. Finally, two wires were joined to the contact pad of every single chip with conductive epoxy (Conductive Epoxy, CW2400-A, USA) so the electrical signal could be sent to the electrodes to generate electric field in the DEP chip.

Figure 4.2: Schematic diagram of the microfluidic channel a) cross-sectional view and b) the top view of the real microfluidic chip.

Figure 4.3: The assembly of the DEP chip with total three layers. All the dimensions are in mm.

4.3 Preparation of Bacterial Samples

In this project, the final targeted particle to be tested is the bacteria commonly found in the water medium including *Escherichia Coli* (*E. coli*) and *Enterococcus Faecalis* (*E. faecalis*). Water is considered to be polluted by human or animal waste when *E. coli* and *E. faecalis* bacteria are found or detected in it. Thus it is always critical to be able to detect those bacteria, especially in a quick and easy way. However due to safety consideration during the experiment, the strain for the above two bacteria was chosen to be a non pathogenic type in this research.

4.3.1 Brief Introduction to Bacterial Samples

All the bacteria can be basically grouped into two major groups, which are called Gram-positive and Gram-negative. Gram staining of the bacteria is usually the first step to categorize the bacteria sample to the two different groups. After a series of staining procedure, the Gram-positive group of bacteria appears in purple colour whereas the Gram-negative group of bacteria appears in pink colour. The main differentiation of the colour shown is due to the difference in properties of bacteria cell wall. *E. coli* is a type of Gram-negative bacteria and *E. faecalis* is a type of Gram-positive bacteria. Figure 4.4 below shows the image of the two bacteria after the gram staining process.

Figure 4.4: Microscopic images on the gram staining of a) *E. coli* and b) *E. faecalis*.

A basic understanding on the bacteria growth is important in order to ensure that the bacteria has a similar behavior each time the bacteria is prepared and ready for experiment testing, thus to ensure the consistency and the accuracy of the experiment result. The following Figure 4.5 shows the general bacteria growth curve.

Figure 4.5: Bacterial growth curve.

The growth for the bacteria is defined by the increase of the number of cells rather than the increase of cells size. This is because in the biological experiment, it is much more convenient and simple to calculate the number of cells than to measure the change of cells size. Once the cells grow to a certain size, division from one cell to two cells will start to occur, the reproduction of the bacteria cells is called binary fission and the process is much simpler than the cell division occurred in eukaryotic cells.

The growth of the bacteria can be divided into four different phase as referred to above figure, namely 1 = Lag phase, 2 = Log phase, 3 = Stationary phase, 4 = Death phase. During the lag phase, the number of bacteria does not increase as can be seen in the above figure; the major change for the bacteria in this phase is the increment of the bacteria size. The lag phase will occur under the condition when medium is changed from

one to another new medium for the bacteria, for example from one nutrient agar to another nutrient agar or from nutrient agar to nutrient broth. After transferring the bacteria from one medium to another new medium, the bacteria would need to take some time to adapt to the new environment, therefore there would be no increment of bacteria population in this phase. However for the log phase, it would be a totally reverse situation as the bacteria cells division starts to occur in this phase, and the bacteria population increase rapidly. In phase 3, which is stationary phase, the rate of bacteria cell division is equal to the rate of bacteria cell death, thus the total bacteria population remain constant in this phase. During the log phase, most of the nutrient is consumed by the bacteria itself, causing the concentration of the nutrient to be reduced and thus the bacteria cell begins to die due to lack of nutrient. The next phase that follows is the death phase. In this phase, the population of the bacteria cell drops drastically. The bacteria which were prepared and tested in this project are under the log phase.

E. coli lives in the human and animal intestinal tracts and they are categorized under the fecal coliforms types of bacteria, which mean they can be found inside the human or animal waste. Therefore, if *E. coli* is found in the drinking water or other water medium source, it would mean that the water has been polluted by the human or animal waste. *E. coli* comes with rod shape with about 2 μm long and 0.5 μm radius. There are a lot of different types or strains for *E. coli*. Due to the safety reason when conducting the experiment, the harmless *E. coli* K12 strain was chosen for testing in this research. *E. coli* K12 is much safer to handle as compared to other toxic *E. coli* strains such as O157:H7 strains, which can cause death. Besides that, *E. coli* K12 can be grown much faster than other types of microbes, once sufficient nutrient is provided. They can be easily cultured

inside the lab. Whereas for *E. faecalis*, it is a type of Gram-positive bacteria, thus it has a very thick cell wall on the outer layer of the membrane. It usually appears as a pair or in a short chain when viewed under microscope. One special characteristic about *E. faecalis* is that it is able to survive in a lot of harsh environment like a high temperature environment. *E. faecalis* lives in the intestinal tracts of human and animal. Hence, when they are found in the water medium, it means the water medium has been polluted by the human or animal waste. The strain chosen for the *E. faecalis* here is ATCC 29212. The following Figure 4.6 shows the SEM images of these two bacterial species taken by other researchers.

Figure 4.6: SEM images for a) *Escherichia Coli* and b) *Enterococcus Faecalis*.

4.3.2 Culturing Steps of Bacterial Samples

Before the culturing of bacteria can be done, all the items used for bacteria culturing such as pipette tips and conical flasks need to be sterilized. This is to ensure that all the items used are free of bacteria and to prevent any unnecessary contamination of

other type of bacteria during the culturing. Safety and cleanness is important during the biological experiment because the microbe that one handles maybe harmful. Besides that, if contamination of other bacteria occurs, it will definitely affect the accuracy of the experiment as the water medium for the experiment will consist of a mixture of some other irrelevant types of bacteria. Therefore, it must always be reminded to maintain the cleanness during the culturing.

The very first step to culture the bacteria basically start with the preparation of the nutrient medium such as nutrient agar to grow the bacteria. The nutrient agar (Oxoid Limited, UK) which is a type of polymer consists of the subunits of sugar galactose will provide sufficient nutrient for the division of bacteria to occur. 14 g of agar nutrient which was in powder form was first dissolved in 500 mL of DI water inside a bottle; the bottle was then heated until the temperature reached near the boiling point or around 60 to 70 °C using the Thermolyne Cimarec 3 hotplate stirrer. While heating, a clean magnetic stirrer was immersed inside the bottle. This magnetic stirrer will be rotated in a very high rotational speed to stir the liquid by the means of rotating magnetic field produced by the hotplate stirrer. During the heating, the cap of the bottle should not be tightly closed to prevent the pressure inside the bottle from building up. For the culture of *E. faecalis*, different type of agar was used and it was called Tryptic Soya Agar (Oxoid Limited, UK).

The next step was to sterilize the nutrient agar. The cap of the bottle was loosened, and then was covered by aluminum foil before the bottle was placed inside the autoclave for sterilization. In this project, Sanyo Labo autoclave was used. Autoclave is used to heat up the aqueous solution beyond their boiling point. In this device, high pressure is incorporated, thus the boiling point of the aqueous solution will be increased and therefore

enable the sterilization to be achieved at higher temperature which makes sure the entire contaminated organism to be killed. For the autoclave device used in this culturing, the whole process took around 2 h and the highest temperature set was 120 °C for 20 min. As high pressure and high temperature were built up during the sterilization, it is always a good practice to wait until the temperature to drop to around 50 to 60 °C before autoclave lid was opened and the bottle was removed from the autoclave for the subsequent steps.

The nutrient agar in aqueous form was then poured into Petri dishes. Care must be taken in order to prevent the nutrient agar to be solidified while it was still inside the bottle. It should be noted that the nutrient agar will be solidified when the temperature falls below around 40 °C. After the nutrient agar solution in the Petri dishes has been solidified, they were covered up by the lid and wrapped with the parafilm wrap. The Petri dishes were then left overnight in the Sanyo incubator with 37 °C. The main objective to leave those Petri dishes in the incubator for 24 h is to ensure that there is no contamination happen inside the Petri dishes before the next step can be carried on. If there is any contaminations happen, the preparations of the nutrient agar have to be redone. Once it was confirmed that there were no contamination, new strain of bacteria was spread on the fresh new nutrient agar with inoculating loop in a zigzag manner. The Petri dishes were then left to incubate inside a 37 °C incubator again for another 24 h.

After 24 h of incubation, the surface area of the fresh nutrient agar would be occupied by new grown bacteria (white in colour) as shown in following Figure 4.7. The Petri dishes with new grown bacteria were then stored in the 4 °C fridge since at this temperature, the metabolism rate for the bacteria was the lowest.

Figure 4.7: Colonies of *E. coli* K12 formed on the nutrient agar plate.

The concentration of bacteria on the nutrient agar can be estimated by the numbers of colony-forming unit or in short called CFU. One CFU would be referred to one isolated colony on the surface of nutrient agar and this isolated colony is formed when one bacteria cell starts to grow and divide into a higher population.

The bacteria have to be suspended in a medium in order to run a testing. Therefore before any testing, the bacteria in the agar plate have to be inoculated into a nutrient broth. Lysogeny broth or Luria-Bertani broth (Becton, Dickinson and Company, NJ, USA) was prepared by dissolving 12.5 g of powder form in 500 mL of DI water inside a bottle before it went through the sterilization process inside the autoclave. For *E. faecalis*, another type of broth was suggested and it was Brain Heart Infusion broth (Oxoid Limited, UK). Once the nutrient broth was ready, fresh 20 mL broth was poured into a universal tube. In practice, 1 CFU of bacteria from the nutrient agar was inoculated and mixed thoroughly with the nutrient broth inside the universal tube. The tube was then put into a shaker (Excella E24 Incubator Shaker Series, New Brunswick Scientific, NJ, USA) for 24 h under 37 °C. The shaker will not only shake the universal tube in a constant frequency, but

also provide a heat temperature. This optimum environment will ensure the bacteria to grow to a higher concentration efficiently. After 24 h of incubation inside the shaker, 100 μ L of nutrient broth consist of bacteria was then transferred to another fresh 20 mL of nutrient broth inside the universal tube. The tube was then left to incubate again inside the shaker for another 3 to 4 h before the experimental testing in order to ensure that all the bacteria tested later on will be in the log phase. However, there is no fixed and restricted way on how to culture the bacteria. The main important thing is to maintain the consistency of the culturing method. By maintaining the same culturing method every time throughout the project, the bacteria will have as close as possible the same behavior properties after each culturing process to yield a more accurate results when tested on the DEP chip each time.

The medium of the bacteria has to be changed from the nutrient broth into the water before the bacteria can be tested in the DEP chip. 1 mL of broth medium was then transferred into micro-centrifuge tube with pipette and rotated in a high speed of 8000 rpm for 5 min with centrifuge machine (Kubota 1120 centrifuge, Kubota Co., Japan). This was to cause the sedimentation of bacteria into the bottom of the micro-centrifuge tube in order to separate them from the nutrient broth as shown in following Figure 4.8. The bacteria was then washed twice with DI water with the same centrifuge machine and vortex machine and finally suspended in 1 mL of DI water. By adopting the culturing method mentioned above, the concentration of the bacteria was measured to be 10^7 cells/mL using the hemocytometer.

Figure 4.8: Sedimentation of E. coli K12 at the bottom tube after centrifugal spinning.

4.3.3 Graph of Absorbance versus Bacterial Concentration

Besides using the hemocytometer to determine the bacterial concentration, spectrophotometric measurement was adopted too in this project to estimate the bacterial concentration upon culturing of the bacteria and before experiment. A standard curve of absorbance versus concentration has to be generated for different bacterial species before the measurement. Compared to hemocytometer, the spectrophotometric measurement is much more accurate since the reading is measured by spectrophotometer, however it is only limited to the measurement of high concentration. Even though the preparation of the standard curve can be very tedious as it require some incubation steps, but once the standard curve has been generated, the measurement of the bacterial concentration can be very fast and simple compared to hemocytometer where the sample to be measured just has to be put inside the spectrophotometer and the concentration can be measured in just single-click.

0.01 mL of bacterial sample from the original sample was transferred with pipette into another micro-centrifuge tube filled with 0.99 mL DI water hence causing 10^{-2} dilution. This micro-centrifuge tube was then shaken by vortex machine to distribute the bacteria equally in the suspension and break up any clumps. The same step applied for the preparation of 10^{-4} , 10^{-6} , 10^{-8} dilutions. The bacteria in each of these four suspensions were then transferred to two agar plates with inoculating loop and then allowed to incubate for 24 h. At the end of the incubation, those Petri dish with bacteria colonies stayed in between 30 to 300 colonies were selected and to be averaged and counted. The number of colonies counted from the Petri dish was then divided by the dilution and volume to yield the final bacterial concentration (in the unit of cfu/mL) of the original sample.

After the bacterial concentration of the original sample has been determined, 0.5 mL of the original sample suspension was transferred into another micro-centrifuge tube filled with 0.5 mL DI water and was then shaken by vortex machine hence causing 1/2 dilution. 1/4, 1/8 and 1/16 dilutions were prepared using the same method. These five different suspensions including the original sample were then poured into the cuvettes, and their absorbances were measured with spectrophotometer respectively under the wavelength of 600 nm. With the absorbance value of the known bacterial concentration obtained from the spectrophotometer, a graph of the absorbance versus concentration can then be finally plotted. Figure 4.9 and 4.10 below show the final standard curve for the *E. coli* and *E. faecalis*.

Figure 4.9: Standard curve of absorbance versus plate count number of E. coli.

Figure 4.10: Standard curve of absorbance versus plate count number of E. faecalis.

4.4 Preparation of Non-viable Bacteria

The non-viable bacteria were prepared by immersing the micro-centrifuge tube with bacteria inside the water bath for 15 min at 80 °C. The water samples was then spread on the surface of the agar plate by using inoculating loop and incubate for 24 h at 37 °C in order to ensure all the bacteria were killed during in the water bath.

4.5 Preparation of Yeast Cells

Yeast cell are a type of microorganism with the shape varied from oval to round and with a diameter of around 3 to 4 μm . 3 g of instant yeast particles were dissolved with 500 mL DI water inside the glass bottle. The bottle was then placed on an orbital shaker to mix uniformly for 15 min at 150 rpm. Like bacteria, the medium need to be changed to fresh DI water too for yeast cell before they were tested in the DEP chip. They were washed twice with the centrifuge and vortex machine before finally suspended in 1 mL of DI water. The concentration of the yeast cell was measured to be 10^7 cells/mL using the hemocytometer.

4.6 Preparation of Polystyrene Beads

Due to the high concentration of polystyrene bead (Polysciences, Inc.) from the original package purchased, the polystyrene beads need to be diluted with DI water before the experiment testing. This is because the high concentration of polystyrene beads will block the view of the electrode image during the observation under the microscope regardless of the volume quantity. After several trials of dilution with DI water and

observation under the microscope, it was finally decided to add on a total amount of 3200 μL DI water into a single droplet of polystyrene beads from the original package, which was equivalent to 23 μL . The calculation of the final concentration is presented below in percentage of the polystyrene bead droplet, which is used in the experiment testing.

Composition of ingredients as implied on the material safety data sheet:

1. Benzene, ethenyl-, homopolymer – 2.5%
2. Water – 97.5%

Therefore the total amount of homopolymer and water in 23 μL droplet is:

$$\text{Benzene, ethenyl-, homopolymer} = 23 \mu\text{L} \times 2.5\% = 0.575 \mu\text{L}$$

$$\text{Water} = 23 \mu\text{L} \times 97.5\% = 22.425 \mu\text{L}$$

After dilution (addition of 3200 μL DI water),

$$\text{The total amount of water is} = 22.425 \mu\text{L} + 3200 \mu\text{L} = 3222.425 \mu\text{L}$$

$$\text{And the amount of homopolymer remained} = 0.575 \mu\text{L}$$

Thus the final concentration in percentage is:

$$(\text{Amount of homopolymer} / \text{Total amount}) \times 100\%$$

$$= [0.575 \mu\text{L} / (3222.425 \mu\text{L} + 0.575 \mu\text{L})] \times 100\%$$

$$= 0.0178\%$$

Therefore, it means that the concentration of the polystyrene beads is diluted from 2.5% to 0.0178% in order to get a clear view under microscope and yet still sufficient for particle trapping and release to occur on the DEP chip. Hemocytometer is used to calculate the concentration of the beads in the solutions and it was found that the concentration was around 136000 beads/ μL after a series of dilution made.

4.7 Staining of Bacterial Samples

In order to differentiate and view the live and dead bacteria simultaneously under the fluorescent microscope, a LIVE/DEAD BacLight Bacterial Viability Kits (Molecular Probes) was used. These kits consist of SYTO 9 green-fluorescent nucleic acid stain and the red-fluorescent nucleic acid stain, propidium iodide. SYTO 9 stain will penetrate both the live (intact membrane) and dead cell (damaged membrane) and will reflect in green colour when view under the fluorescent microscope. Whereas propidium iodide is only able to penetrate those with damaged membrane (dead cell) and will reflect in red colour under the fluorescent microscope. The two stains with ratio 1:1 (1.5 μ L respectively for both stains) were added into the 1 mL water medium consisted of the mixture of live and dead cells. It was then left to incubate for at least 15 min in dark at room temperature. The mixture of live and dead cells stained in green and red colour respectively is shown in the following Figure 4.11. Care has to be taken to prevent the stains from the exposure of light during the experimental testing as it may reduce its sensitivity or the emission strength of the stains.

Furthermore, in order to differentiate the Gram-positive and Gram-negative bacteria simultaneously under the fluorescent microscope, a different staining kit was used. It is called LIVE BacLight Bacterial Gram Stain Kits (Molecular Probes). These kits consist of SYTO 9 green-fluorescent nucleic acid stain and the hexidium iodide red-fluorescent nucleic acid stain. The staining protocol is the same as the LIVE/DEAD BacLight Bacterial Viability Kits.

Figure 4.11: Mixture of live (green) and dead (red) cells when viewed under the fluorescent microscope.

4.8 Summary

The DEP chip had been successfully fabricated in the clean room utilizing the microfabrication method. Two types of bacterial sample, namely *E. coli* and *E. faecalis* were prepared with conventional microbiological method and other sample such as yeast cell was prepared too. The staining of the live and dead bacteria was performed in order to differentiate them inside the DEP chip during the experimental testing.

CHAPTER 5

Study on Bacteria Trapping and Separation

5.1 Introduction

After the fabrication of the DEP chip and the preparation of the bacterial and other samples, the experimental testing is now ready to begin. The main objective of this testing is to study and understand the trapping behavior of the neutral particle in the DEP chip which consists of electrode arrays. The trapping behavior between non-living microorganism (polystyrene bead) and living microorganism (bacteria) in the DEP chip were compared and the separation between these two different neutral particles was achieved using the same DEP chip. The trapping efficiency of this DEP chip was evaluated too with hemocytometer. Two different bacterial species which include *Escherichia coli* and *Enterococcus faecalis* were then tested. The main differences between these two bacteria are their cell wall structure and internal properties, and this may cause the effective dielectric properties to be slightly different. Different experiment conditions and parameters were set in the experiment in order to study the differences in their dielectrophoretic behavior and dielectrophoretic force experienced by both species. Besides that, the separation efficiency was evaluated for different mixtures of neutral particles under the effect of voltage amplitude, frequency and flow rate. The separation was achieved by adopting the flow separation method.

5.2 Experimental Setup

The DEP chip was connected to a function generator (AFG3000 Series Arbitrary/Function Generators, Tektronix, Inc., USA) in order to generate an AC electrical signal. The frequency and amplitude of the applied voltage can be easily altered with this function generator. An oscilloscope (TDS3000 Series Digital Phosphor Oscilloscopes, Tektronix, Inc., USA) was used to verify the AC signal provided by the function generator. A syringe pump (Model NE-1000, New Era Pump System Inc., NY, USA) was used to precisely control the flow rate and pump the sample medium continuously into the DEP chip via a 1ml capacity syringe (BD 1 ml Syringe Luer Lock Tip, Cole-Parmer, Inc. Co., IL, USA). The DEP chip was mounted onto the stage of a conventional microscope (Olympus BX41) with CCD (charge-coupled device) camera. The experimental setup is shown in the following Figure 5.1.

Figure 5.1: Schematic diagram of the experimental setup.

5.3 Bacteria Trapping Characterization in Continuous Flow

5.3.1 Bacteria Trapping Behavior in the DEP Device

When the bacteria were injected and flown pass the DEP chip which consists of electrode arrays, the bacteria were trapped on the electrode edges especially at the electrode tips which have the highest electric field strength, under the positive DEP. And with the continuous pumping of bacterial sample using the syringe pump, more bacteria were flowing into the DEP chip, and they were trapped continuously and joined to those bacteria which have been trapped on the electrode edges, and formed a pearl-chain liked formation. The length of the pearl-chain was increasing as more bacteria were trapped, until it reached and touched the opposite electrode tip. However, during the trapping, not only one pearl-chain was formed, but several of them at single electrode tip. When this huge amount of pearl-chains linked up together and reached the opposite electrode tip, the phenomena of “DEP Bridge” can be observed as shown in Image 3 in Figure 5.2.

In the DEP chip, it consists of total 59 electrode arrays. And each electrode array has a gap distance of 30 μm (electrode tips to tips). The length of the pearl-chain for every electrode array was increasing as the flow continued, till it reached the opposite electrode tip forming DEP Bridge at the first array, followed by the second and subsequent arrays. Finally the entire electrode gaps at its electrode tips were filled with DEP Bridge. The reason the bacteria filled up the electrode gaps in this sequence were because most of the bacteria have been trapped at the electrode tips of the first few arrays as they flowed pass the front part of the DEP chip, leaving lesser amount of bacteria to flow towards the end part. When the trapping of bacteria in one array has been “saturated” (forming DEP Bridge), the rest of the bacteria would flow pass this array and trapped at its next array

forming another DEP Bridge there. This bacterial trapping behavior in the continuous flow manner is shown in the following Figure 5.2.

Figure 5.2: Microscopic image of Escherichia coli bacteria trapping at the first and second electrode arrays of the DEP chip under the experimental operating parameters of 1 MHz frequency, 10 V_{peak-peak} voltage amplitude and 1 μL/min flow rate [14].

However, all the phenomena described above will only happen provided the voltage amplitude supplied is high enough. If the voltage amplitude is set to be too low, the length of the bacterial pearl-chain will remain short and will not reach the opposite electrode to form DEP Bridge no matter how long the supply of the bacterial sample into the DEP chip, instead it will stick to the condition like the one shown in Image 2 in Figure 5.2. This is because the DEP force will be reduced if the voltage amplitude is set to be very low. Hence, it is only able to trap small amount of bacteria at the electrode tips and produce shorter length of pearl-chain since it is well-known that the DEP force is directly proportional to the voltage amplitude.

It can be seen from the above Figure 5.2, no bacteria were trapped at the beginning when there was no voltage supplied. However, when the voltage was turned on for 30 seconds, some bacteria were getting started to be trapped and accumulated at the electrode edges especially at the electrode tips in the pearl-chain liked formation. And at this point, it is not considered as a DEP Bridge yet, but rather termed as “incomplete DEP Bridge” since the two electrodes opposite to each other has not been fully joined with the bacterial pearl-chains. 1 min after the continuous flow of bacterial suspension, it can be seen that the first array has formed the DEP Bridge while the second array was still on the process of forming. However, the second array would eventually complete the formation of the pearl-chain if the bacterial suspensions continue to be supplied, and it was followed by the subsequent arrays until the whole DEP chip consist of DEP Bridge at every electrode tips.

Figure 5.3 below shows the schematic diagram of the trapping behavior under the high and low voltage amplitude. In between the high and low voltage amplitude, there is a value called “threshold voltage”. When the voltage amplitude is set above or equal to this

threshold voltage, DEP Bridge can be formed whereas when the voltage amplitude is set below this threshold voltage, DEP Bridge will not form.

Figure 5.3: Trapping behavior of bacteria in the continuous flow manner under the high and low voltage amplitude [14].

Since the electrode here is opaque and black in colour, thus those bacteria that located on top or on the electrode surface is not viewable under the inverted microscope. Therefore, in order to ensure that all the bacteria were trapped on the electrode gap but not on the electrode surface. SYBR Green Staining (Molecular Probes) was used to stain the bacteria in order to observe its position of trapping. And from the microscopic image

obtained as shown in the following Figure 5.4, it has been confirmed that all the bacteria were trapped on the electrode gap forming the DEP Bridge.

Figure 5.4: Confirmation of all the bacteria trapping on the electrode gap rather than on the electrode surface by the help of SYBR Green Staining.

5.3.2 Dielectrophoresis Characterization between *E. coli* and *E. faecalis*

All the experimental parameters were set to be the same in order to compare the dielectrophoresis difference between these two bacterial species. Unlike the living organism and non-living organism which have an obvious difference in their dielectric properties, different bacterial species exhibit nearly the same dielectric properties among themselves. Therefore, it hardly find the difference in their cross over frequency. One way to evaluate the difference in DEP behavior between those two bacterial species was to investigate if there was any difference in DEP force experienced by them. It would not be ideal to just purely test a single bacterial cell but rather to adopt continuous trapping method on huge amount of bacterial concentration in order to evaluate the difference in

DEP force. This is because different live bacterial species may not only exhibit a close cross over frequency, the DEP force experienced by them are also near to each other. Thus, by adopting continuous trapping method, the DEP force experienced by different bacterial species would be magnified and their difference could be easily distinguished.

It has been explained in that if the voltage amplitude was too low, no DEP Bridge can be formed. Thus at certain point of minimum voltage amplitude, or threshold voltage, the DEP Bridge can be formed. Different flow rate will produce different hydrodynamic drag force, and thus the threshold voltage will be varied accordingly in order to overcome the drag force and form the DEP Bridge. And it is believed that the flow rate will be directly proportional to the threshold voltage since higher DEP force is required to overcome the higher hydrodynamic drag force. Therefore, based on this phenomenon, both the *E. coli* and *E. faecalis* were tested separately to find out the threshold voltage at different flow rate. Five different flow rate was decided and set, they were 0.5, 0.8, 1, 1.2, 1.5 $\mu\text{L}/\text{min}$. At different respective flow rate, the peak to peak voltage amplitude was increased in a step of $0.5 V_{\text{peak-peak}}$ in order to determine the threshold voltage and the frequency was remained the same at 1 MHz.

5.3.2.1 Difference in Threshold Voltage for *E. coli* and *E. faecalis*

The experiment result shows that the overall threshold voltage for *E. coli* is higher than *E. faecalis* besides at the flow rate of 0.5 $\mu\text{L}/\text{min}$ as shown in Figure 5.5. This means that higher voltage amplitude is required for *E. coli* to form the DEP Bridge compared to *E. faecalis* or in other words, at the same voltage amplitude, *E. faecalis* tend to experience higher DEP force compared to *E. coli*. It is known that the polarization of

the surface charges will affect the DEP force. And the “surface” for *E. coli* and *E. faecalis* is different in their structure whereby *E. coli* has a three layer surface of membrane- thin wall-membrane and *E. faecalis* has a two layer of membrane-thick wall. However it is believed that the most dominate factor for the difference in DEP force experienced by them is the internal properties of the cells due to the application of high frequency. This is because at 1 MHz, the frequency is high enough to penetrate the membrane which acts as a capacitance into the cell interior. And it is known that Gram-positive (*E. faecalis*) bacteria consist of more K^+ ion thus causing them to have higher internal conductivity and more polarizable compared to Gram-negative bacteria (*E. coli*) under the same electric field strength. Another main reason that contributes to the lower threshold voltage needed for *E. faecalis* to build the DEP Bridge is because of the difference in electric field strength of the neighboring particles. It has been explained from the numerical simulation result that particle with higher polarizability (or higher permittivity) will have a higher sudden increment in the electric field strength on the particle’s outer boundary and hence able to produce higher DEP force. Therefore the nearby particle can be easily attracted and form the DEP Bridge. From Figure 5.5, it also shows that the relationship between the threshold voltage and the flow rate was linearly related since the adjusted R-square was close to 1.

Figure 5.5: Difference in the threshold voltage under different flow rate for *E. coli* and *E. faecalis* [14]. (Adjusted R-Square>0.99)

5.3.2.2 Difference in Threshold Voltage for Viable and Non-viable Bacteria

Figure 5.6 and 5.7 show that the non-viable bacteria for both species have a higher threshold voltage compared to their viable one. Or in other word, these imply that non-viable species experience lesser DEP force compared to viable species under the same operating condition. It has been studied by other researchers that when bacteria undergo heat treatment process, the dielectric properties of the bacteria will be altered, and hence its CM factor changes. When the CM factor is changed, the DEP force will be indirectly be modified according to general expression of dielectrophoresis equation. It is believed that the membrane of the cell is perforated following the process of heat treatment, and ion in the cytoplasmic can leak out through the perforated membrane to the medium causing the conductivity of the cytoplasmic to be reduced near to the medium. It has been proven that at this 1 MHz operating frequency, the viable bacteria experience

much stronger positive DEP force than non-viable bacteria and the results shown further justify this. However, it could be the other case at the other frequency range due to the difference in the CM factor. There is another interesting phenomenon shown in Figure 5.6 and 5.7. The difference in threshold voltage between the viable and non-viable cells for *E. coli* is much larger than *E. faecalis* at specific flow rate. It could be attributed to the structure difference for Gram-positive and Gram-negative bacteria since it is known that Gram-positive (*E. faecalis*) has much thicker cell wall and this cell wall is believed to act as a shield and protection for the cells and minimize the damage or perforation of cell membrane caused by heat treatment process.

Figure 5.6: Difference in the threshold voltage under different flow rate for viable and non-viable *E. coli* [14]. (Adjusted R-Square>0.90)

Figure 5.7: Difference in the threshold voltage under different flow rate for viable and non-viable *E. faecalis* [14]. (Adjusted R-Square>0.95)

5.3.2.3 Difference in Threshold Voltage for Bacteria in Three Different Days after Preparation

Figure 5.8 shows the difference in threshold voltage for the same *E. coli* species at three different days. The species were tested upon preparation by centrifuge process and thus it is considered as Day 1 sample. The same species was then left overnight for 24 h (Day 2) and 48 h (Day 3) at light environment under the room temperature, and were tested again to determine their threshold voltage. It was found that the threshold voltage was different for these 3 days. Day 3 has the highest threshold voltage among the three different samples and it means that it experiences the lowest DEP force compared to Day 1 and 2. One of the main reasons could be due to the decay rate of the bacteria because bacteria would decay or died in a relatively high amount everyday in the water if lack of nutrient. Therefore in Day 2 and 3, it is believed that huge amount of bacteria would have

died due to the insufficient of nutrient in the water medium and there would be a mixture of live and dead bacteria. And it is known from Figure 5.6 and 5.7 that non-viable or dead bacteria would experience lesser DEP force, thus causing the threshold voltage of *E. coli* suspension in Day 2 and 3 to be relatively higher. Another possible reason could be due to the fact that the metabolism of the bacteria causes the loss of ion from cytoplasm into the environment, thus affecting the internal conductivity and hence the CM factor of the bacteria.

The determination of the threshold voltage to form the DEP Bridge for different bacterial species under different conditions allow the accurate prediction of the differences in DEP force experienced by them. This method of DEP force prediction is relatively easy and fast compared to other tedious analysis and it provides useful information for other relevant separation works.

Figure 5.8: Difference in threshold voltage under different flow rate for the same *E. coli* suspension at three different days [14]. (Adjusted R-Square>0.95)

5.4 Separation of Bacteria from Other Neutral Particles

With the same DEP chip, separation of bacteria from other neutral particles such as polystyrene bead and yeast cell can be achieved. The experimental setup remained the same as that mentioned in the above section. In dielectrophoresis, there are a number of different methods as described in Chapter 2 to achieve the separation, for example flow separation, field-flow fractionation, stepped flow separation and traveling wave separation. In this section, flow separation method was adopted to study and analyze the separation performance for the three different mixtures: *E. coli* – yeast cell, *E. coli* – polystyrene beads and yeast cell – polystyrene beads. The separation efficiency of these three mixtures were evaluated and compared under the same experimental parameters.

The main purpose of this experiment was to characterize the separation efficiency using the flow separation method under the effect of voltage, frequency and flow rate. The voltage and frequency can be set using the function generator while the flow rate can be set using the syringe pump. Before the experiment, the concentration of the sample with the mixture of two different particles were counted using hemocytometer, the sample was then pumped into the DEP chip with a syringe pump. After several minutes, a droplet of the sample was formed at the output of the DEP chip, and it was then transferred by a pipette into the hemocytometer again to calculate the sample's outlet concentration. Finally, the separation efficiency can be calculated using the following formula.

$$\eta = \left| \frac{c_{outlet}}{c_{inlet}} - \frac{c'_{outlet}}{c'_{inlet}} \right| \times 100\% \quad (5.1)$$

In which c and c' are two different types of particles. The subscript of *outlet* represents the

particle concentration after the particle has flown pass through the DEP chip and collected in the output whereas the subscript *inlet* represents the particle concentration before it was injected into the DEP chip.

The following Figure 5.9a shows the microscopic image of the sample mixture of *E. coli* – yeast cell from the hemocytometer before they were injected into the DEP chip. The larger size is the yeast cell and the smaller dot size is *E. coli*. Figure 5.9b, c, d show the microscopic image of the sample collected in the output with the voltage amplitude of $1 V_{\text{peak-peak}}$, $5 V_{\text{peak-peak}}$, and $10 V_{\text{peak-peak}}$ supplied on the DEP chip respectively.

Figure 5.9: a) Microscopic image of the hemocytometer for the mixture of *E. coli* and yeast cell before injected into the DEP chip. b) Sample collected on the channel output which has flown through the DEP chip with $1 V_{\text{peak-peak}}$, c) $5 V_{\text{peak-peak}}$ and d) $10 V_{\text{peak-peak}}$ supplied voltages.

It can be seen that after the sample has passed through the DEP chip with 1 $V_{\text{peak-peak}}$ supplied voltage, there were less yeast cell collected in the output compared to the input whereas the concentration of the bacteria remain almost unchanged, or in other words, more yeast cell have been trapped in the DEP chip, leaving *E. coli* to flow pass the electrode array and flow towards the channel outlet. However, at 5 $V_{\text{peak-peak}}$, it can be seen that the concentration of both species have been greatly reduced, indicating that both species were trapped simultaneously on the DEP chip. Finally at 10 $V_{\text{peak-peak}}$, there were no single particles collected in the channel outlet, thus indicating that both species were trapped 100% simultaneously. Thereafter, based on the specific formulation given by the manufacturer of the hemocytometer to calculate the concentration, and with Equation 5.1, the separation efficiency can be calculated. The concentrations of each particle were counted three times in the hemocytometer before their average concentrations were substituted into Equation 4.1. The separation efficiency for other mixtures under different effects is shown on the following sections.

5.4.1 Effect of Voltage Amplitude on the Separation Efficiency

Figure 5.10: Separation efficiency with respect to voltage amplitude for different particle mixtures at frequency of 1 MHz and flow rate of 1 $\mu\text{L}/\text{min}$.

Figure 5.10 shows the separation efficiency of three different particle mixtures under different voltage amplitude. The experimental result shows that the separation efficiency of the E.coli – yeast cell mixture reducing as the voltage amplitude is increased (inverse proportional) whereas both the separation efficiency of E.coli – polystyrene bead and yeast cell – polystyrene bead mixtures are increasing when the voltage amplitude is increased (direct proportional). As depicted in Figure 5.9b, at $1 V_{\text{peak-peak}}$, the concentration of yeast cell has reduced on the channel output whereas the concentration of E.coli remained almost the same for the separation of E. coli – yeast cell. This indicates that the separation has been highly achieved and its efficiency reaches around 76% according to Figure 5.10. However, at $5V_{\text{peak-peak}}$ for E. coli – yeast cell, the separation efficiency only achieves around 13%. And it can be seen on Figure 5.9c that there were only small amount of E.coli and yeast cell appeared on the channel output, indicating that both species have been trapped concurrently on the DEP chip with not much separation occurred. At $10V_{\text{peak-peak}}$ for E. coli – yeast cell, the separation efficiency reduces even lower and it reaches 0% since there were no any single species appeared on the channel output as shown in Figure 5.9d, indicate that both species have been all trapped on the DEP chip yielding no separation at all.

The reason that the separation efficiency drops as the voltage increases for E. coli – yeast cell mixture is mainly due to the size difference of the species. The size of the yeast cell is typically 3 to 4 times larger than E. coli, and this will contribute to the difference in gravity force experienced by them. Yeast cell with larger size will experience much higher gravity force than bacteria. At $1 V_{\text{peak-peak}}$, the DEP force is too low to trap either yeast cell or E. coli. However, the reason why there is still decrease in yeast cell

concentration on the channel output as shown in Figure 5.9b is because they were trapped by gravity force instead of DEP force. And *E. coli* which experience a much smaller gravity force due to its smaller size were continued to be flushed out by the hydrodynamic drag force. At 5 $V_{\text{peak-peak}}$ and 10 $V_{\text{peak-peak}}$, both species started to experience relatively high positive DEP force in 1 MHz frequency, therefore both species were trapped simultaneously on the DEP chip without any separation hence causing the separation efficiency to drop drastically.

As for *E. coli* – polystyrene bead and yeast cell – polystyrene bead mixtures, the main reason why the separation efficiency increases when the voltage amplitude is increased can be due to obvious difference in their dielectric properties. It has been investigated that polystyrene bead hardly experience positive DEP in a wide range of frequency, thus it can easily cause the separation to occur if another particle experience positive DEP. Due to this reason, the separation efficiency is greatly affected by the effect of voltage amplitude on both the *E. coli* and yeast cell only. Both mixtures reach 100% separation efficiency at 10 $V_{\text{peak-peak}}$ and it can be further justified from the microscopic image shown in Figure 5.11 where for the *E. coli* – polystyrene bead mixture, all *E. coli* were trapped on the electrode tips but all the beads were flowing continuously towards the channel outlet. However, the only difference is the efficiency value at lower voltage (The separation efficiency for *E. coli* – polystyrene bead mixture is around 20% and yeast cell – polystyrene bead is around 76% at 1 $V_{\text{peak-peak}}$). This can be again due to the above mentioned reason in which yeast cell experience higher gravity force, thus it can achieve high separation efficiency even at low voltage amplitude (where DEP force is negligible) for yeast cell – polystyrene bead mixture. But for *E. coli* – polystyrene bead mixture, the

separation efficiency is purely affected by the trapping capability of *E. coli* since the gravity force is negligible for *E. coli*. Thus, when the voltage amplitude is increased, the trapping of *E. coli* will be increased (due to high DEP force), and hence increase the separation efficiency.

Figure 5.11: Separation of *E. coli* and polystyrene beads at frequency 1 MHz, voltage amplitude $10 V_{\text{peak-peak}}$ and flow rate $1 \mu\text{L/min}$. The beads appeared to be a circular bright colour dot compared to the bacteria with dark black colour [14].

5.4.2 Effect of Frequency on the Separation Efficiency

When discussing about the effect of frequency on the separation efficiency, the parameter of CM factor has to be studied and analyzed because CM factor is dependent on the frequency. The CM plot or variation of the CM factor on a specific range of frequency has to be plotted out in order to understand the dielectrophoresis behavior of a certain particle. It is particularly important to take note on the frequency value (cross over frequency) where the transition of CM factor from positive to negative value or vice-versa happens since it will decide on whether the particle will experience a positive or negative

DEP. The CM plot for the viable bacteria (*E. coli*) and viable yeast cell is shown in the following Figure 5.12. The biological particle has a complicated internal structure and therefore, a concentric multi-shell model is used to model the cell in order to yield the CM plot of the biological particle.

Figure 5.12: Real part of the CM factor for viable bacteria, *E. coli* and yeast cell.

Figure 5.13: Separation efficiency with respect to frequency for different particle mixtures at voltage amplitude of $10 V_{\text{peak-peak}}$ and flow rate of $1 \mu\text{L/min}$.

Figure 5.13 shows the separation efficiency of three different particle mixtures from the frequency range of 1 MHz to 10 MHz. The trend of the graph obtained is totally reverse compared to the result obtained on the effect of voltage amplitude where now *E. coli* – yeast cell mixture is directly proportional to the frequency whereas *E. coli* – polystyrene bead and yeast cell – polystyrene bead mixtures are inversely proportional to the frequency. Since the applied voltage and flow rate is constant, the only parameter which affects the DEP force and hence the separation efficiency is the real part of the CM factor according to the dielectrophoresis expression because this parameter is dependent on frequency. For the *E. coli* – yeast cell mixture, the separation efficiency is increasing with the increase in frequency. This is due to the reason that the real part of the CM factor for *E. coli* is dropping drastically in the range of 1 MHz to 10 MHz according to the CM plot shown in Figure 5.12 whereas for yeast cell, the CM factor stays almost constant in this frequency range with just a minor increment from 0.3 to 0.4. Thus the DEP force which is experienced by *E. coli* is reducing while the force experienced by yeast cell stays almost constant. At 1 MHz, even though the CM factor for yeast cell is almost the half of the bacteria (which will reduce the DEP force), but the larger size of yeast cell will contribute to a higher DEP force due to direct proportionality relationship of the particle volume and DEP force. Furthermore, yeast cell will experience much larger gravitational force than bacteria. Due to these reasons, both species were totally trapped at 1 MHz, causing the separation efficiency to be 0%. However, as the frequency increase, there was more and more *E. coli* collected in the output due to the reduction of the CM factor and hence the reduction of DEP force, leaving the yeast cell to be continued trapped in the DEP chip, hence the separation efficiency is increasing. For both the *E. coli* – polystyrene

bead and yeast cell – polystyrene bead mixtures, the separation efficiency is very high at the beginning, because the polystyrene bead was flowing out without trapping whereas *E. coli* and yeast cell were trapped in the DEP chip. As the frequency was increased, more *E. coli* was flushed out (due to lower DEP force) together with the polystyrene bead causing the separation efficiency to reduce drastically. For yeast cell, due to the nearly constant DEP force experienced by it throughout the frequency range from 1 MHz to 10 MHz, the separation efficiency stays almost constant and reaches 100% efficiency.

5.4.3 Effect of Flow Rate on the Separation Efficiency

Figure 5.14 below shows the separation efficiency of three different particle mixtures under different flow rate. It can be seen that the flow rate has little or no effect on the separation efficiency as there is not much changes of efficiency along the flow rate from 0.5 to 3 $\mu\text{L}/\text{min}$.

Figure 5.14: Separation efficiency with respect to flow rate for different particle mixtures at voltage amplitude of 10 $V_{\text{peak-peak}}$ and frequency of 1 MHz.

It can be seen that the separation efficiency for the three mixtures are almost constant. The separation efficiency of *E. coli* – yeast cell mixture is 0% at lower flow rate but there is slight increment as the flow rate is increased. Whereas for *E. coli* – polystyrene bead and yeast cell – polystyrene beads mixtures, the separation efficiency reach 100% at lower flow rate but there are slight decrement as the flow rate is increased. When the flow rate is changed, the hydrodynamic drag force will be varied. The difference in drag force experienced by different size particle is relatively smaller compared to the difference in gravity force experienced by different size particle according to the their respective equation. Therefore the difference in flow rate contributes only minor variation in the separation efficiency.

5.4.4 Conclusion on Separation Efficiency

The experimental results on the separation efficiency obtained above provide important information on the different effects on the separation performance based on the flow separation method. Among the three common effects (voltage amplitude, frequency and flow rate) used in the general dielectrophoresis experiment, it was found that the flow rate has the least or no effect on the separation efficiency whereas the voltage amplitude and frequency play a major role on the separation behavior. Thus, one has to pay much attention to the voltage amplitude and frequency rather than flow rate in order to achieve the best separation performance. The experimental results also show and explain the difference in separation efficiency between similar dielectric properties (both biological particle) but different size particle (*E. coli* – yeast cell) and different dielectric properties

(one biological and another non-living particle) but similar size (E. coli – polystyrene beads).

5.5 Separation of Live and Dead Bacteria

Separation of live and dead bacteria was achieved using the same DEP chip. In order to achieve this separation based on the flow separation method, the CM plot for both the live and dead bacteria has to be plotted and is shown in the following Figure 5.15. And once again concentric multi-shell model is used to model the cell. It can be seen that both type of species exhibit positive DEP at lower frequency range but transit to negative DEP at higher frequency range. However, it can be seen that the cross over frequency for both species are different and thus by setting the experimental frequency in between the cross over frequency for the live and dead bacteria, the separation could be achieved and is shown in Figure 5.16 below.

Figure 5.15: Real part of the CM factor for live and dead bacteria with the frequency range from around 8 MHz to 30 MHz to achieve separation.

Figure 5.16: Separation of live and dead bacteria. Live bacteria (stained green) were trapped along electrode edge and dead bacteria (stained red) were flowing continuously towards the channel outlet.

5.6 Effect of AC electroosmosis on Bacteria Motion

AC electroosmosis is another kind of AC electrokinetics phenomena besides dielectrophoresis. It causes a fluid motion over the surface of electrode, which in turn causes the particle movement. AC electroosmosis is highly dependent on the frequency and there is a certain specific frequency value (usually occurred at around kHz range) where the fluid velocity reaches the maximum. The fluid velocity caused by the AC electroosmosis is the maximum on the electrode edge and it reduce gradually towards the inner surface of the electrode, and this fluid flow will move the particle and accumulate it on the center of the electrode surface. Therefore, AC electroosmosis could be considered as one of the method for particle trapping besides dielectrophoresis. However, if only

dielectrophoresis (especially positive DEP) but not any other AC electrokinetics phenomena is adopted to trap particle, attention has to be paid on the frequency setting because at certain frequency, there will be an overlapping of AC electroosmosis over positive DEP. For example bacteria species, according to the CM plot shown in Figure 5.15, it will exhibit steady positive DEP from 1 kHz to 1 MHz range. However, it is known that AC electroosmosis usually occur around kHz range and hence it will overcome positive DEP and be a more dominant factor since it has been studied that for a similar size of particle, the velocity caused by the DEP is an order of magnitude less than the velocity caused by AC electroosmosis. Therefore the main objective of this part of experiment is to identify the frequency where AC electroosmosis phenomena will happen for bacteria since this phenomenon is undesirable in this project and they are shown in the following Figure 5.17.

300 kHz

300 kHz

300 kHz

300 kHz

Figure 5.17: AC electroosmosis phenomenon of *E. coli* bacteria at various design of electrode. The circles in the diagram show the position of the bacteria.

In order to be able to view the bacteria on the electrode surface, an upright microscope was used instead of inverter microscope. A droplet of water medium consist of bacteria was then dropped onto the electrode surface with a pipette, no syringe pump was required. The voltage amplitude was set to be $10 V_{\text{peak-peak}}$, and the frequency was initially set at 1 MHz. At this frequency, the bacteria exhibit positive DEP and hence it can be seen that the bacteria were trapped along the electrode edges as shown in the left image of Figure 5.17. The frequency was then being reduced gradually while monitoring the bacteria behavior on the electrode surface. And when it reached around 300 kHz, all the bacteria which are initially trapped on the electrode edges were being moved towards the electrode surface instantaneously and trapped there as shown in the right image of Figure

5.17 due to the AC electroosmosis process. Noted that the acting frequency of AC electroosmosis was the same for the bacteria at various design of electrode and this further justify that the acting frequency of AC electroosmosis for bacteria is 300 kHz.

In order to verify the distinctive AC electroosmosis behavior of the bacteria with other neutral particle, polystyrene bead was tested under the same experimental condition. Figure 5.18 shows the transformation from positive DEP into AC electroosmosis phenomenon for polystyrene bead.

Figure 5.18: AC electroosmosis phenomenon of polystyrene bead at various design of electrode. The circles in the diagram show the position of the bead.

It can be seen that the polystyrene beads were trapped and accumulated on the electrode edges at 1 MHz. Unlike the trapping behavior of bacteria which were trapped in a pearl-chain manner, the polystyrene beads were seen to be trapped in a chunk of group manner as shown in the left image of Figure 5.18. Like bacteria, the polystyrene beads exhibited AC electroosmosis behavior too when the frequency was reduced to a certain value. As observed under the microscope, the acting frequency of AC electroosmosis for polystyrene beads is 10 kHz, which is different from the bacteria. At this frequency, it can be seen that the beads were moved by the fluid and finally accumulated on the surface of electrode as shown in the right image of Figure 5.18. In this simple experiment, it can be concluded that every neutral particles regardless of living or non-living organism have their own distinctive AC electroosmosis acting frequency. And the electrode geometry has little or no effect on the acting frequency.

5.7 Summary

The bacterial trapping behavior in the DEP chip under the continuous flow manner has been investigated and studied. The ability to form the “DEP Bridge” has been utilized as a new method to distinguish the DEP force experienced by different bacterial species, namely *E. coli* and *E. faecalis*. The threshold voltage to form the DEP Bridge for these two different bacterial species has been found and discussed. Besides that, the separations among different neutral particles were achieved utilizing the flow separation method, one of the general strategies to achieve separation via dielectrophoresis. And their separations performance or separation efficiency were compared and characterized based on the effect

of voltage amplitude, frequency and flow rate. Finally, AC electroosmosis effect on the bacteria was studied too.

CHAPTER 6

Characterization of Bacteria Trapping Using Impedance Measurement

6.1 Introduction

The experimental results on the bacterial trapping behavior under the continuous flow manner from the previous chapter showed that bacteria are able to grow the pearl-chain and form DEP Bridge during positive dielectrophoresis. And due to this special characteristic, an impedance measurement will be adopted to measure the impedance of the water medium consists of bacteria with respect to time. When bacteria form a pearl-chain and eventually led to the formation of DEP Bridge, it will fill the electrode gap and touch on the opposite nearby electrode. This gap filling by the bridge will definitely reduce the impedance of the whole DEP chip. In this impedance measurement, two experimental setups were proposed. One utilized the current probe incorporated with oscilloscope while the other utilized the impedance analyzer method. And it was proven that both methods were able to detect the impedance changes with respect to time. Different operating parameter such as voltage amplitude will yield a different impedance magnitude signal which is directly related to the trapping behavior of bacteria in the DEP chip. With this impedance measurement, the bacterial concentration in the water medium as well as the percentage of bacterial mixture can be characterized in a real time manner.

6.2 Equivalent Circuit for Impedance Measurement in Bacteria Trapping

When bacteria were trapped on the electrode, it would contribute to the changes of the impedance magnitude. And an equivalent circuit was used in order to have a better understanding on how the trapping of bacteria would affect the impedance magnitude. As shown in the following Figure 6.1, the circuit basically can be divided into two parts with both having the parallel connection of resistance, R and capacitance, C . The first one is due to contribution of electrode and the medium solution (R_E and C_E), and the second one is due to the contribution of the bacteria trapping behavior (R_D and C_D). Attention is paid on the latter part because the impedance of the electrode and medium solution would always stay constant if the same electrode and same medium solution are used throughout all the experiments.

Figure 6.1: Equivalent electrical circuit for the bacteria trapping on the electrode.

R_E and C_E would remain constant, and attention is paid on R_D , the total resistance of DEP Bridge. Assuming each DEP Bridge would consist of n rows of pearl-chain and each row of pearl-chain would consist of m bacterium. The expression of total resistance for DEP Bridge is shown below:

$$R_D = \frac{mR_B + R_W}{n} \quad (6.1)$$

where R_B is the resistance of a single bacterium and R_W is the resistance of the water.

Noted that when there is a complete formation of DEP Bridge that link up the opposite electrode, the term R_W will be vanished in Equation 6.1 and R_D would only depend on the resistance of the bacterium since electrical current can flow directly from one electrode to its opposite electrode through the bacteria. However, if the formation of DEP Bridge is incomplete, the electrical current has to flow pass the water after finish flowing pass the incomplete DEP Bridge in order to complete a close circuit and therefore the resistance of water, R_W has to be considered in this case.

The resistivity of DI water (the suspension medium of bacteria in this project) can be as high as around 5000 Ωm [58]. The resistivity of DI water is much higher when compared to the resistivity of bacteria with around 10 Ωm (consider only its main electrical properties in the cytoplasm) [58]. Thus when substituting the value into the Equation 6.1 and comparing the resistance for both the complete DEP Bridge and incomplete DEP Bridge (compare only the numerator of Equation 6.1 by assuming the n , number of the pearl-chain is the same for both the phenomena), it is found that the R_D for incomplete Bridge ($mR_B + R_W$) will always be higher than R_D for complete Bridge (mR_B).

That is because even though m for incomplete Bridge is always smaller than complete Bridge (the maximum m for complete Bridge can reach for *E. coli* is around 15 due to the electrode gap of 30 μm and *E. coli* length of 2 μm , and the preferred longitudinal DEP trapping orientation), the value of $mR_B + R_W$ (incomplete Bridge) will still always be larger than the value of mR_B (complete Bridge) due to the reason of more than 100 times higher in resistivity for DI water compared with the bacteria. In conclusion, no matter how long the DEP Bridge is, it will always have a higher resistance than the complete DEP Bridge unless it grows long enough to reach the opposite electrode.

6.3 Experimental Setup for Impedance Measurement

There are two methods proposed for the impedance measurement in this project. One of them utilizes the current probe and another one utilizes the impedance analyzer. The one which utilize the current probe has a relatively simple experimental setup compared to the impedance analyzer method. But by using the current probe, the impedance magnitude cannot be obtained in a real time manner since some calculation has to be made before obtaining the final impedance magnitude. However, for impedance analyzer method, the impedance magnitude can be obtained in a real time manner even though the experimental setup is relatively complicated.

6.3.1 Impedance Measurement with Current Probe

Current probe is an electrical device which is used to measure the electrical current via the oscilloscope (TDS3000 Series Digital Phosphor Oscilloscopes, Tektronix, Inc., USA). In this project, a CT-6 High Frequency AC Current Probe (Tektronix, Inc., USA)

was used. This type of current probe enables the measurement of electrical current in a very high frequency range (up to 2 GHz). In the experimental setup, the signal from the function generator (AFG3000 Series Arbitrary/ Function Generators, Tektronix, Inc., USA) was connected to the Channel 1 of the oscilloscope via BNC cable, and this Channel 1 displayed the voltage amplitude supplied to the DEP chip. As for the current probe, it was connected to the Channel 2 of the oscilloscope from the DEP chip in order to display the electrical current signal output from the DEP chip. The oscilloscope was then connected to the PC using the appropriate RS-232 cable, and finally all the signals (peak to peak voltage amplitude and current) were displayed and recorded by software called WaveStar (Tektronix, Inc., USA) in the PC. By having both the peak to peak value of voltage amplitude ($V_{\text{peak-peak}}$) and current ($I_{\text{peak-peak}}$) recorded with the software, the impedance magnitude can be calculated by simply dividing the $V_{\text{peak-peak}}$ with $I_{\text{peak-peak}}$. The experimental setup with current probe as well as the connection between the current probe and DEP chip is shown in the following Figure 6.2.

Figure 6.2: Experimental setup for the impedance measurement with current probe.

6.3.2 Impedance Measurement with Impedance Analyzer

With impedance analyzer, the impedance magnitude will be directly displayed on the computer automatically. Besides that, the impedance analyzer will produce more data point (impedance value) in a shorter period. However, the experimental setup for this method is relatively complicated. Some electrical circuit needed to be designed in order to decouple the current of the power supplied (function generator) from the current of the measurement signal (impedance analyzer). In this research, Solartron SI 1260 impedance analyzer (Advanced Measurement Technology, Inc., UK) was used. The following Figure 6.3 shows the experimental setup for the impedance measurement utilizing impedance analyzer.

Figure 6.3: Experimental setup for the impedance measurement with impedance analyzer.

Noted that from the above figure, there were two additional electronic components (capacitor and inductor) mounted in the experimental setup. The function of these two components was to decouple the signal from the function generator and the signal from

the impedance analyzer. Capacitor allows high frequency signal to pass through but impede low frequency signal. Whereas inductor allows low frequency signal to pass through but impede high frequency signal. The following Figure 6.4 shows the two different workspaces to conduct the impedance measurement with impedance analyzer. One of the workspace focus on the DEP experiment which involve equipments such as microscope, syringe pump and function generator. Whereas the other workspace focus on the impedance measurement and the main equipment involved is the impedance analyzer with the PC.

Figure 6.4: Two workspaces required for conducting the impedance measurement utilizing the impedance analyzer method.

The frequency from the function generator was set at 1 MHz as usual (which cause the positive DEP for the bacteria) and this high frequency current from the function

generator was only restricted to move towards the DEP chip and not towards the impedance analyzer due to a connection of inductor (which impede high frequency signal) in front of the impedance analyzer. As for the impedance analyzer, the measurement frequency was set to low frequency, 2 kHz. This was to ensure that the current from the impedance analyzer did not flow towards the function generator due to a connection of capacitor (which impede low frequency signal) in front of the function generator, but only towards the DEP chip to measure its impedance. In the experimental setup, capacitors with 100 nF and inductors with 100 μ H were chosen.

6.4 Experimental Results from Impedance Measurement

The main objective for this part of experiment is to use the impedance measurement to detect the change of impedance value in a real time manner when there are bacteria existing in the water medium. Different conditions were attempted in the experiment to demonstrate how the impedance changes corresponding to various conditions.

6.4.1 Impedance Test under Constant DEP Voltage

Using the impedance analyzer method and the appropriate circuit design as shown in the Figure 6.3, the impedance magnitude of the DEP chip could be measured and is shown in the following Figure 6.5. Before the measurement started, the water with *E. coli* bacteria was pumped through the DEP chip at the flow rate of 1 μ L/min. The function generator and the impedance analyzer were then turned on concurrently. Upon turning on the function generator, the impedance measurement was done automatically by the impedance analyzer. The voltage amplitude for the electrodes on the DEP chip was set at

12.5 V_{peak-peak}, and the measurement was run continuously for 1 h.

Figure 6.5: Impedance magnitude of the DEP chip with respect to the time at voltage amplitude of 12.5 V_{peak-peak} and flow rate of 1 μ L/min.

From the result obtained, it can be seen that the impedance magnitude decreases in a steady manner with the time. There is a sudden increment of impedance at the beginning. This could be due to the transient behavior of the impedance analyzer at the beginning. However, the most important observation is that the signal (impedance) from the DEP chip is changing continuously, this implies that some phenomena has happened and kept happening in the DEP chip. In order to investigate the phenomena happening in the DEP chip that cause the changes of impedance magnitude signal, images on the DEP chips' front, center, and end part (defined according to the water flow direction) were taken after 5, 15, 30, and 45 min of experiment and they are shown in Figure 6.6.

Water flow direction

After 5 minutes:

Front:

Pearl-chain started to form on the electrode edges.

Bacterial pearl-chain

Center:

No bacteria were trapped on the electrode edges.

End:

No bacteria were trapped on the electrode edges.

After 15 minutes:

Front:

More and more pearl-chains were formed at the electrode edges, DEP Bridge were formed eventually.

Center:

There was still no trapping of bacteria yet.

End:

There was still no trapping of bacteria yet.

After 30 minutes:

Front:

The growth of DEP Bridge reached its maximum.

Center:

Formation of DEP Bridge started.

End:

There was still no trapping of bacteria yet.

After 45 minutes:

Front:

The growth of DEP Bridge reached its maximum.

Center:

The growth of DEP Bridge reached its maximum.

End:

The growth of DEP Bridge reached its maximum.

Figure 6.6: Experimental images taken at 5, 15, 30 and 45 min at the front, center and end part of the DEP chip during the impedance measurement.

After 5 min, some bacteria were seen trapped on the electrode tips at the front part of the electrodes, and at the mean time the impedance magnitude of the DEP chip had reduced as shown in Figure 6.5. However, no bacteria were trapped at the center and end part of electrodes yet after 5 min. After 15 min, bacterial pearl-chains continued to grow longer and longer at the front part of electrodes and the DEP Bridge were eventually formed. Still no bacteria were trapped at the center and end part of electrodes. And at this 15 min mark, the impedance magnitude was further reduced. After 30 min of experiment, the center part of electrodes began to form the DEP Bridge but there was still no trapping of bacteria yet at the end part of electrodes. At this point, the impedance magnitude continued to reduce since more and more DEP Bridges were formed in the DEP chip. However, after 45 min of experiment, it could be seen that the entire electrode gaps were filled with DEP Bridge. And from this point onwards, the impedance magnitude started to be remained constant as shown in Figure 6.5. This could possibly due to the reason that not much bacteria could longer be trapped as all the electrode gaps had been filled with DEP Bridge, thus the impedance magnitude were not affected.

With this experimental result, it can be seen that the trapping of bacteria, especially the formation of DEP Bridge do affect the impedance magnitude. And thus the changes of electrical signal (impedance magnitude) can act as a real time indicator to indicate if the water medium contains bacteria or not.

More experiments were done in order to further validate the relationship between the bacteria trapping and the impedance magnitude by applying different voltage amplitude to the same DEP chip but the same flow rate and frequency. They were 4, 8, 10, 11 $V_{\text{peak-peak}}$. Figure 6.7 below shows the impedance magnitude with respect to time under

different voltage amplitude. The impedance variation in term of percentage was expressed rather than the value of impedance magnitude itself for comparison. This was due to the reason that the impedance magnitude of the DEP chip at the beginning may be slightly varied each time it was tested. It could possibly due to some slight contamination on the electrode surface or there might be some bacteria residue left over after each time of experiments. However, the difference was calculated to be not more than 3% for the same DEP chip for each testing. And most importantly, more attention was paid on the variation of impedance magnitude (how the trapping of bacteria would affect the reduction rate of the impedance magnitude) rather than the exact value of the impedance magnitude. The impedance variation was calculated based on the formulation shown in Equation 6.2 below.

$$\text{Impedance Magnitude Variation} = \frac{\text{Final Impedance Magnitude} - \text{Initial Impedance Magnitude}}{\text{Initial Impedance Magnitude}} \times 100\% \quad (6.2)$$

Figure 6.7: Comparison of impedance magnitude variation under the different voltage amplitude.

From the above graph shown in Figure 6.7, it could be seen that the higher the voltage amplitude supplied to the DEP chip, the greater would be the final impedance variation for example $12.5 V_{\text{peak-peak}}$ can record up to around – 12% compared to $8 V_{\text{peak-peak}}$ which record up to around – 6%. All the impedance magnitude variations are shown negative value due to reason of the continual reduction in the impedance magnitude value. The main reason for the direct proportional behavior for the voltage amplitude and the impedance variation is because of the ability to trap bacteria for the DEP chip. The higher the voltage amplitude, the higher will be the DEP force, and hence able to trap higher amount of bacteria. As more bacteria are trapped and form the DEP Bridge, the overall impedance magnitude of the DEP chip will be definitely reduced. In order to further verify the direct relationship between the trapping of bacteria and the impedance variation, a $0 V_{\text{peak-peak}}$ was applied to the DEP chip. With $0 V_{\text{peak-peak}}$, it means the bacteria will experience no DEP force, hence no trapping will occur. And 0% variation is recorded at this voltage amplitude as shown in Figure 6.7, or in other words, there is no reduction in impedance magnitude since no trapping of bacteria can occur.

Another interesting observation shown in the experimental result is that all the curve generally posse exponential reduction characteristic. It means that the reduction rate is gradually decreased until at some specific time, the reduction will halt (impedance magnitude variation become constant). Furthermore, it can be noticed that the “specific time” when the impedance variation start to become constant for every voltage amplitudes are different. Those “specific time” are called saturation time thereafter. The higher the voltage amplitude, the saturation time gets longer for example $12.5 V_{\text{peak-peak}}$ has around 3000 s saturation time, $10 V_{\text{peak-peak}}$ with around 2500 s and $4 V_{\text{peak-peak}}$ with around 1500 s

as shown in Figure 6.7. The main reason that causes the above phenomena to happen is due to the difference in trapping ability for different voltage amplitude. Higher voltage amplitude will have a greater trapping ability, hence it will have more room to trap higher amount of bacteria and form more DEP Bridge, and in order to complete more DEP Bridge, longer time is definitely be required. For lower voltage amplitude with weaker trapping ability, there will be certain limit in the amount of bacteria trapped due to lower DEP force. Beyond its limitation, the DEP chip no longer trap any bacteria even though the water medium with bacteria is still being pumped continuously, therefore shorter time is required to trap relatively lower amount of bacteria and hence producing shorter saturation time. In conclusion, it has been proven that the impedance magnitude has a direct relationship with the trapping behavior in the DEP chip. From the information obtained in the impedance variation curve (information such as maximum variation and saturation time), one can easily depict and predict the trapping behavior of the bacteria in the DEP chip in a real time manner.

6.4.2 Effect of Bacterial Concentration on Impedance

Upon having the understanding of the relationship between the impedance magnitude and the bacterial trapping behavior, more experiments were done. One experiment was to verify if the DEP chip was able to detect the difference in bacterial concentration or not by producing different impedance magnitude signal. It is always an important task for every microbiologist to be able to tell the bacterial concentration level especially in the fastest possible way. However, with the help of the microfluidic technology combined with the powerful tool of electrical technology such as impedance

measurement, the time required to quantify the bacterial concentration can be greatly reduced. The timing could be possibly reduced from one day (with conventional microbiological method) to just within an hour (with microfluidic technology). The following Figure 6.8 and 6.9 show the capability of the DEP chip to produce different impedance magnitude signal with respect to different bacterial concentration for *E. coli* and *E. faecalis* using the current probe measurement method.

Figure 6.8: Impedance magnitude variation for different bacterial concentration of *E. coli*.

Figure 6.9: Impedance magnitude variation for different bacterial concentration of *E. faecalis*.

It can be seen from the above figures that the higher the bacterial concentration, the greater will be the impedance variation for both bacterial species. The curves for both species have a similar trend where the concentration of 10^6 cells/mL can reach up to around – 15% of variation for E.coli and around – 18% for E. faecalis. When the concentration level is reduced to 10^5 cells/mL, it can be seen that the impedance variation for the concentration level of is relatively low (only – 2% for E. coli and – 3% for E. faecalis). However, at 10^4 cells/mL, there is no change at all in impedance magnitude, the impedance variation stay constant at 0% which mean that the DEP chip is not able to detect this bacterial concentration level or below. From this experimental result, it proves that the DEP chip in this project is able to differentiate the concentration level of the bacteria but with the minimum detection level of 10^5 cells/mL.

During the measurement, an observation was made on the bacterial trapping behavior via the microscope. For 10^6 cells/mL and after 30 min of continuous trapping, it was observed that all the electrode arrays in the DEP chip had formed the DEP Bridge for E. coli whereas only 23 out of total 59 electrode arrays had formed the DEP Bridge for E. faecalis. For 10^5 cells/mL and after 30 min of continuous trapping, it was observed that 13 electrode arrays in the DEP chip had formed the DEP Bridge for E. coli and only 5 electrode arrays had formed the DEP Bridge for E. faecalis. Finally for 10^4 cells/mL, it was observed that not a single electrode arrays had formed DEP Bridge for E. coli and E. faecalis. From the above observation, it is now better to explain the reason why the higher the bacterial concentration, the greater will be the impedance variation. That is due to direct proportionality of the number of DEP Bridge with the impedance variation.

Moreover, there is one interesting phenomena observed during the measurement. It has been concluded in the previous chapter that *E. faecalis* tend to experience higher DEP force than *E. coli*, but during the experiment here, more DEP Bridge were formed for *E. coli* compared with *E. faecalis* which sound unreasonable. However, if more attention is paid on the “width” of the DEP Bridge, one can notice that the width of the DEP Bridge for *E. faecalis* is much wider than *E. coli*, this means that at every electrode arrays in the DEP chip, more bacteria have been trapped for *E. faecalis* which explain why even though *E. faecalis* experience stronger DEP force but form the DEP Bridge in a lesser electrode arrays. The following Figure 6.10 further explains the different phenomena observed for both bacterial species after 30 min.

Figure 6.10: Particle which experience lower DEP force (*E. coli*) as shown on the left image will have thinner DEP Bridge but form at more electrode arrays. Particle which experience higher DEP force (*E. faecalis*) as shown on the right image will have wider thickness of DEP Bridge even though the Bridge form at relative lesser electrode arrays.

6.4.3 Effect of Bacterial Mixture on Impedance

Besides predicting the bacterial concentration of a single species in the water medium as shown in the previous section, the DEP chip is able to predict the different concentration percentage of the bacterial mixture too with the help of the DEP Bridge

formation phenomena. The water sample in the real world usually contains more than one type of bacterial species, and they may exist in different concentration. Some species may be harmful to human when consume, and some other may not, therefore it is important to be able to identify the percentage (or concentration) of a certain bacterial species especially those that are harmful among the rest of the remaining harmless bacterial species. For conventional microbiological method, it may require immunological method to identify the type of bacteria which exist in the water medium, in which the preparation procedure is tedious and also time consuming. However, by using the DEP chip in this project, no separation is required but still can be able to detect the different proportion of bacterial mixture in a real time manner with very short duration by adopting the DEP Bridge formation phenomena. Based on the finding and observation from the previous chapter, it was found that different bacterial species will exhibit different ability to form the DEP Bridge under the same conditions (same voltage amplitude and flow rate). And it has been explained earlier that when DEP Bridge is formed, it will greatly reduce the impedance magnitude of the DEP chip compared to those without formation of DEP Bridge (even though there is still trapping of bacteria on the electrode tip). Therefore, by combining the above two phenomena, it makes the detection of different proportion of bacterial mixture possible. If there is higher proportion of bacteria species which experience higher DEP force exist in the water medium, DEP Bridge would form after some specific time and hence there will be higher impedance variation whereas if there is higher proportion of bacteria species which experience lower DEP force exist in the water medium, DEP Bridge would not form after the same period of time and hence there will yield a lower impedance variation.

As a demonstration purpose, *E. coli* and *E. faecalis* mixture with different percentage proportion (concentration) in the water medium were prepared and were tested at different voltage amplitude in order to verify if different signal of impedance magnitude could be produced. Before the experiment, equal concentration of *E. coli* and *E. faecalis* were prepared each with 1 mL volume quantity. Thereafter, 0.8 mL of *E. coli* and 0.2 mL of *E. faecalis* were pipette into a micro-centrifuge tube and then mixed thoroughly which yield a water sample medium consist of 80% *E. coli* and 20% *E. faecalis*. The same steps applied to the preparation of water sample medium consist of 20% *E. coli* and 80% *E. faecalis*. The water samples were then tested in the DEP chip at voltage amplitude of 4 $V_{\text{peak-peak}}$ and 5 $V_{\text{peak-peak}}$ respectively. Different impedance magnitude signals were produced and their impedance variations were shown in the following Figure 6.11.

Figure 6.11: Impedance magnitude variation for different proportion (percentage) of bacterial mixture in a single water medium tested at different voltage amplitude.

For the mixture of 80% *E. coli* and 20% *E. faecalis* which tested at $4 V_{\text{peak-peak}}$, it can be seen from the Figure 6.11 that it produced the lowest impedance variation (-4.5% at the 15 min mark). When observed under the microscope, no DEP Bridge was formed, and hence it produced a relatively lower impedance variation. However, under the same voltage amplitude of $4 V_{\text{peak-peak}}$, another bacterial mixture with 20% *E. coli* and 80% *E. faecalis* was tested and it can be seen that it produced a relatively higher impedance variation (-7.5% at the 15 min mark). No DEP Bridge was formed too for this sample. Therefore, the only explanation on why there are still 3% difference on the impedance variation even though both are not forming DEP Bridge is because *E. faecalis* tend to experience higher DEP force compared to *E. coli* (as proven in the previous chapter), and thus there would be more total number of bacteria (regardless of the types) trapped on the electrode edge or electrode tip for the mixture with 20% *E. coli*, 80% *E. faecalis*.

At $5 V_{\text{peak-peak}}$, the bacterial mixture of 80% *E. coli* and 20% *E. faecalis* produced an impedance variation of -10% at the 15 min mark whereas the bacterial mixture of 20% *E. coli* and 80% *E. faecalis* produced an impedance variation of -19% which mean there was a 9% difference for both samples. When observed under the microscope, the former one did not form the DEP Bridge whereas the latter one did form the Bridge. This explain why the difference in the impedance variation for both sample is higher at $5 V_{\text{peak-peak}}$ (9% difference) compared to $4 V_{\text{peak-peak}}$ (3% difference). Figure 6.12 below shows the schematic diagram of the difference in trapping behavior for both the bacterial mixture of 80% *E. coli*, 20% *E. faecalis* and 20% *E. coli* and 80% *E. faecalis* at $5 V_{\text{peak-peak}}$. *E. coli* were stained in green colour whereas *E. faecalis* were stained in orange colour using LIVE Baclight Bacterial Gram Stain Kits.

Figure 6.12: Schematic diagram of the trapping behavior for the bacterial mixture of a) 80% *E. coli*, 20% *E. faecalis* and b) 20% *E. coli* and 80% *E. faecalis* at $5 V_{\text{peak-peak}}$. *E. coli* were stained in green colour whereas *E. faecalis* were stained in orange colour.

The schematic diagram shown in the Figure 6.12 was exactly what was happening when observed under the fluorescent microscope. It can be seen that there was no DEP Bridge formation for the bacterial mixture of 80% *E. coli*, 20% *E. faecalis* (left image of Figure 6.12) whereas there was DEP Bridge formed for the bacterial mixture of 20% *E. coli* and 80% *E. faecalis* (right image of Figure 6.12) at $5 V_{\text{peak-peak}}$ (more real fluorescent microscopic image can be seen in the following Figure 6.13). Therefore these explain why there is a great difference in the impedance magnitude variation for the two bacterial samples.

Besides that, there was an interesting phenomenon observed during the testing. For the bacterial mixture of 80% *E. coli*, 20% *E. faecalis*, even though there were more *E. coli* in the water sample, it seemed that the total amount of bacteria trapped on the electrode edges had an equal amount ratio of 1:1 for the *E. coli* and *E. faecalis*. Whereas for the bacterial mixture of 20% *E. coli* and 80% *E. faecalis*, it can be seen that more *E. faecalis* were trapped at the electrode edges (forming DEP Bridge) compared to *E. coli* which seem more “logical” since there were more *E. faecalis* in the water sample.

Figure 6.13: Fluorescent microscopic image taken during the impedance measurement at $5 V_{\text{peak-peak}}$. (a) The trapping behavior of bacterial mixture of 80% *E. coli* and 20% *E. faecalis* upon applying voltage, (b) After 1 min of applying voltage. (ci) After 2 min of applying voltage and (cii) the trapping behavior of bacterial mixture of 20% *E. coli* and 80% *E. faecalis* after 2 min of applying voltage.

More real images on the bacterial trapping behavior from the fluorescent microscope were captured and shown on the above Figure 6.13. For the bacterial mixture of 80% *E. coli*, 20% *E. faecalis*, it can be seen that both bacterial species were trapped concurrently upon the supplied of voltage as shown in the Figure 6.13 (a). However after 1 min, it can be seen that more and more *E. coli* (green in colour) were trapped compared to *E. faecalis* (orange in colour) as shown in the Figure 6.13 (b), and this was due to the reason of continuous supplied of high concentration of *E. coli* bacteria. After 2 min, the bacterial pearl-chain grew longer but still yet to form the DEP Bridge, and it could be seen that there were some mixture of *E. faecalis* lie in between the bacterial pearl-chain especially in the tips of the chain forming an almost equal amount ratio of 1:1 for the *E. coli* and *E. faecalis* in the pearl-chain as shown in the Figure 6.13 (ci) even though there was a relatively higher amount of *E. coli* compared to *E. faecalis* in this water sample. When compared with the bacterial mixture of 20% *E. coli*, 80% *E. faecalis* at the same voltage amplitude of $5 V_{\text{peak-peak}}$ and after 2 min, it could be seen that the DEP Bridge has been formed and the Bridge comprised almost all the *E. faecalis* where only a very small amount of *E. coli* can be seen as shown in the Figure 6.13 (cii).

The main reason that causes the difference between the trapping behaviors for the bacterial mixture of 80% *E. coli*, 20% *E. faecalis* and 20% *E. coli*, 80% *E. faecalis* is once again due to the relatively higher DEP force experienced by *E. faecalis* compared to *E. coli*. For 80% *E. coli*, 20% *E. faecalis*, the concentration for *E. faecalis* may be much lower, but the DEP force experienced by them is high and thus they can still be seen appeared in almost equal amount with *E. coli* in the bacterial pearl-chain. Moreover, they are most likely to appear in the end portion of the pearl-chain since the longer it goes in

the pearl-chain, the lower will be the electric field strength, and therefore, only *E. faecalis* are capable to be trapped on this lower electric field strength region. For 20% *E. coli*, 80% *E. faecalis*, the DEP Bridge are able to form and it consists of almost all the *E. faecalis* due to higher DEP force experienced by them compared to *E. coli*.

6.5 Summary

Two methods of bacterial detection based on impedance measurement manner have been proposed. Both methods are able to detect the presence of bacteria in the water medium in a real time manner with high accuracy. By adopting the trapping behavior (ability to form the DEP Bridge for different bacterial species) studied in the previous chapter, and combined with the impedance measurement proposed here, the impedance magnitude detection signal can be produced. The detection signals basically interpret the trapping phenomena occur in the DEP chip, and thus it enable the monitoring and understanding of the bacterial trapping behavior in the DEP chip without even have to utilize the microscopic tool but by just analyzing the impedance magnitude signals.

CHAPTER 7

Conclusion and Future Work

7.1 Conclusions

In this research, dielectrophoresis (DEP) was used as a means for bio-particles trapping and detection. The target particles used in this research are live bacteria in the water medium such as *Escherichia Coli* and *Enterococcus Faecalis*. The first reason for choosing these bacteria is that their existence in the water medium indicates contaminated water and causes serious public safety issues. Therefore it is always important to be able to detect their existence in water with the fastest possible way. The second reason is that these two bacterial species are categorized under different bacterial group, namely gram negative for *E. coli* and gram positive for *E. faecalis*. Thus, it is of significant interest and usefulness to investigate the dielectrophoretic behavior of the two different groups of bacteria for possible differentiation of the two types.

Numerical simulation of the electric field distribution for the electrodes in the DEP microfluidic chip was carried out in order to understand how the particles behave in the DEP chip during the experiment. Attention was also paid to the electric field distribution for the neighboring particles as the experiment involved high concentration of particles during the trapping.

The designed microfluidic chip was then fabricated in the clean room using the microfabrication technique. The bacteria were subsequently tested in the DEP chip in a

continuous flow manner. It was found that a DEP Bridge could be formed. Different bacterial species or even the same species but under different condition would have different ability to form the DEP Bridge, and the difference in ability to form the DEP Bridge indicated the difference in DEP force experienced by them.

Next, separation of different neutral particles such as yeast cell, polystyrene bead and bacteria were investigated and their separation performance was indicated by the separation efficiency. For bacteria which experience electric field at low frequency, AC electroosmosis may become significant and cause significant influence on the positive dielectrophoresis phenomena. Therefore it is important to identify the acting frequency for the AC electroosmosis. The acting frequencies for *E. coli* and polystyrene bead were found and compared for different electrode designs.

Finally, the electric impedance technique was combined with the trapping behavior of the bacteria (DEP Bridge formation) to achieve real time detection instantaneously for the presence of bacteria in the water medium, and to provide the bacterial concentration information as well as the percentage mixture of different bacterial species.

7.2 Future Works

Based on the trapping behavior, especially the difference in the ability to form the DEP Bridge under the same voltage amplitude and flow rate, it was proven that different bacterial species experienced different DEP force. Based on this finding, a new DEP chip could be designed and fabricated to achieve the continual separation of different bacterial species. The separation may be achieved by making use of the difference in the DEP force experienced by different bacterial species, which was shown in this work. Therefore the

structure design of the electrodes in the DEP chip is critical in order to create a domain in which different forces could be experienced by different bacteria when they flow through this domain and hence to achieve the separation.

Besides that, by adopting the method mentioned in this thesis to determine the differences in DEP force experienced by the bio-particles, it is suggested that more types of bacterial species should be tested using the same DEP chip in this thesis especially under the same group of bacteria (either gram negative or gram positive bacteria). If the threshold voltage required to form the DEP Bridge is lower, it means the DEP force experienced is higher and vice-versa. Therefore by comparing the threshold voltage of different bacterial species, one can easily compare the DEP forces experienced by different bacterial species. It has been shown in this work that different group of bacteria experiences different DEP force whereby gram positive bacteria (*E. faecalis*) tends to experience higher DEP force than gram negative bacteria (*E. coli*). However, more works can be done in order to validate if the bacteria under the same group will experience different DEP force.

Furthermore, the DEP chip developed in this thesis was demonstrated to be capable of producing different impedance magnitude based upon the trapping behavior of the bacteria. Different concentrations of bacteria were able to produce different impedance magnitude. However, the difference in concentration for the sample tested was relatively large (10^4 cells/mL, 10^5 cells/mL and 10^6 cells/mL). Hence difference in concentration under the same order of magnitude should be tested in future to see if there is still variation in the impedance magnitude. Otherwise, a smaller scale of electrode inside the microfluidic chip can be fabricated to increase its sensitivity and hence increase the

accuracy of the impedance magnitude signal.

Finally, it is suggested that an electrical circuit can be designed for a circuit board and incorporated with other electric device such as LED that can act as a signal indicator for detection. Thus the signal indicator will be able to produce a signal to alarm us in a real time manner whenever the impedance magnitude reaches a certain value or drops to a certain value. By having this complete electrical circuit, impedance analyzer, current probe, oscilloscope or computer systems are not required in the real time warning system. Hence a more complete and ideal lab-on-a-chip system, which can perform various functions, could be produced.

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