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Capacitive detection of single bacterium from drinking water with a detailed investigation of electrical flow cytometry

Mustafa Tahsin Guler[a,*], Ismail Bilican[b]

[a] Department of Physics, Kirikkale University, Kirikkale 71450, Turkey
[b] Science and Technology Application and Research Center, Aksaray University, Aksaray 68100, Turkey

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ABSTRACT

Pathogenic contamination of drinking water is critical in regard to human health. In this study, we investigated the electrical detection of single bacterium from drinking water. A microfluidics chip consisting of polydimethylsiloxane (PDMS) microchannel and gold microelectrodes was fabricated with conventional microfabrication techniques. Electrical characterizations were done with an LCR meter and the measurements were in good agreement with simulation results. The impact of channel and electrode dimensions was studied for the different type and size of particles, using both experimental and simulation techniques. In addition, the effect of excitation signal frequency and solution conductivity was analyzed employing both simulation and experimental methods. Finally, capacitive detection of a single *Escherichia coli (E. coli)* from drinking water was successfully carried out under optimum parameters and design geometries.

1. Introduction

In the past, pathogens were a serious source of threat that endangered the lives of millions of people. Today, the situation is a little brighter by means of advanced medical techniques. However, pathogenic diseases still cause thousands of deaths, even in developed countries. As an example, more than 38 million pathogenic illnesses occur in the USA, resulting in 71,878 hospitalization and 1,686 deaths, annually [1]. The situation is even worse in other parts of the world where about 75% of the people still do not have direct access to clean water supplies [2]. Based on the recent records, water pollution related diseases accounted for approximately 14,000 deaths per day where more than 12,000 of those victims were children under the age of 11. 75% of the deaths were caused by biological contamination of the water, which refers to microorganisms such as bacteria and viruses [2]. These statistics demonstrate the significance of the pathogen detection from drinking water.

There are several methods that have been developed for detecting pathogens [3]. They can be classified according to the detection strategy or sensing technique. Every method has some advantages or disadvantages over each other. Although some techniques can give the exact amount and type of species inside the sample, they suffer from the detection complexity. Conversely, some methods have simple procedures but don't meet minimum sensitivity requirements. Today, the most reliable detection approaches are still based on microbiological methods developed by the great scientists Pasteur and Koch [4], but those techniques need advanced lab equipment along with technical staff. They are also highly time consuming. Recently, rapid detection methods have been introduced to meet current requirements. While some of these methods are simply updated versions of the traditional ones and provide better sensitivity and less time demand, some of them are based on the innovative and popular ideas such lab on a chip (LOC) technology [5,6].

The detection mechanism may be based on the optical, chemical or electrical applications [3,7–9]. Electrical detection which is also employed in this study has two fundamental branches: faradaic and nonfaradaic [10]. Although some methods are based on sensing faradaic current that is caused by the electrochemical reagents inside the solution [11–13], others are based on sensing the nonfaradaic current generated by the push-pull effect of excitation voltage on ions in the solution [14]. Nevertheless, one of the oldest rapid bacteria detection technique, which is still in use and commercially available, is based on evaluating the total conductivity of the solution that tends to increase in presence of pathogens because of the ionic nature of the bacteria metabolites [15,16] While there are some side techniques that is used to increase the sensitivity and

* Corresponding author.
E-mail address: gulermt@gmail.com (M.T. Guler).

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selectivity like antibody tagging [17,18] and dielectrophoresis [19], electrical method offers even higher sensitivity at a single cell level when integrated with microfluidic structures [20,21]. Microfluidics technology enables us to introduce a small amount of the sample to the electrodes through microchannels. Because of the insulator property of the channel materials, it also triggers the generation of a confined electric field, which in turn leads to sensitivity enhancement [22].

However, advancement in microfabrication technology has enabled the microelectrode manufacturing process in research labs [23]. So far, bacteria detection has been performed cumulatively either with direct [24] or indirect ways [25], such that the solution including bacteria is introduced on to microelectrodes and the impedance is measured and shows little shifts according to bacteria concentration. Due to its stationary measurement character, a strong impedance drift, which originates from some physical phenomenon such as temperature fluctuations or electrochemical reactions [26], recorded together with the signal coming from the bacteria leading a rise in noise. Hence, they have poor sensitivity such that the limit of detection (LOD) of these sensors is nearly 10^6 cfu/ml. However, there are only few studies claiming the detection of single bacterium electrically [22,27] and they all employ a microfluidics structure to achieve this. This technique is independent from the drifts and fluctuations of the total signal because of its snap measurement characteristics.

However, these microfluidics chips suffer from a complex fabrication procedure due to the integration of the preferred top-bottom electrodes because of their higher sensitivity as compared to other types. Despite this, in this study, we utilized coplanar electrodes and achieved great sensitivity by means of relatively small electrode and microchannel dimensions. Although there is a publication employing coplanar electrodes, it uses a quake valve structure, decreasing the channel height to enhance sensitivity and avoid clogging, and therefore needs a complex fabrication procedure [28]. Instead, in our approach, we filter the solution using a filter at optimum size allowing the passage of the target particles while hindering the particles that could possibly clog the channel. In this study, microchannels are acquired simply by casting polydimethylsiloxane (PDMS) on the fabricated mold and channel electrode integration is conducted with bolts and nuts. We performed the detection with an LCR meter, which is a much easier bench top device to use in comparison to the lock-in amplifier system. On the other hand, the LCR meter provides an opportunity to compare the experimental results with those of the simulations directly owing to its usable physical output, such as impedance and capacitance. We did a detailed analysis of solution conductivity and frequency in wide range using COMSOL(R) MULTIPHYSICS 5.0 software and found the point where a cell-like particle and bead-like particle can be differentiated. In addition, simulation results are demonstrated visually in terms of electric current stream lines, which are more illustrative to explain the fact. Thanks to the impedance output of the experimental setup, computational results could be compared with experimental ones. Hence the effect of conductivity and frequency is also investigated experimentally. Besides, the particle's positional dependence of impedance peaks is investigated with COMSOL simulations to explain the experimental results. Despite there are some studies that utilize capacitive measurement for detection, they focus on the cumulative static measurement [29]. After determining the optimum experimental parameters, capacitance measurement is done using the LCR meters-specific mode. Finally, under these optimum conditions, we were able to detect a single bacterium analyzing the measured capacitance signal. Thanks to our custom made LabVIEW (National Instruments) code, the electrical results either impedance or capacitance were analyzed real time. This enabled us double checking the electrical output with the microscope camera.

Table 1
Simulation parameters.

Component	Parameter	Value
Cell membrane	Conductivity	2×10^{-6} S/m
	Relative permittivity	5
	Thickness	10 nm
Cell interior	Conductivity	0.6 S/m
	Relative permittivity	60
PS bead	Conductivity	1.2×10^{-9} S/m
	Relative permittivity	2.55
Solution	Relative permittivity	80

1.1. Theory

Although there are different approaches which employ capacitors and resistors to represent certain parts of the solution and cell [30], the AC electro kinetics phenomenon is well-explained with Maxwell mixture theory [31]. However, analytical solutions of theoretical approach to coplanar electrodes and cell-like membranous particles are extremely complex, which pushes us to make our calculations in computational methods. Cells differ from the solid particles in terms of structure leading to an insulator membrane that surrounds its conductive interior. When the medium in which cells are suspended is more conductive than the cell cytoplasm, a cell can't be differentiated from a solid particle electrically. COMSOL simulation results demonstrate this phenomenon clearly as shown in Fig. 1. Here only for these simulations whose results are shown in Figs. 1 and 2, a 2D model was used to make a simple demonstration without sacrificing the physics behind the phenomenon. Hence the circles in those figures actually correspond to a cylinder instead of a sphere. There is no consensus about the cell parameters in the literature and we used the following values which is also consistent with other works as shown in Table 1 [19,32,33].

A cell-like particle that has the same electrical properties with a living microorganism, shares the same electrical characteristics with an opaque bead-like particle when suspended in a highly conductive buffer solution. It can be seen that only few electric field lines can penetrate into the cell-like particle which can be ignored experimentally as shown in Fig. 1b. For an opaque particle, the electric field can't pass through the particle due to the high resistance of the particle as a whole. However, in a cell-like particle, the electric current is blocked by the thin membrane. Although the volumetric ratio of the cell membrane is less than 1/100 in a cell, it blocks the current completely because of the surrounding characteristic structure. Hence the electric current can't reach to the highly conductive cell cytoplasm. This situation is true when the buffer solution is more conductive than the cytoplasm around which the electric current prefers to bypass the cell. As the conductivity of the buffer solution decreases, the electric current can further enter into the cell. This fact can be explained in terms of the less attractive feature of the buffer solution surrounding the cell due to the lower conductance. Therefore, the electric current has more tendencies to pass through the cell because of the conductive cytoplasm as shown in Fig. 1b–f. When the electric current passes through the cell, it carries information about the cell's interior and enables discrimination of the different types of particles like a bead or cell. Fig. 1a and b demonstrate that with a high conductivity buffer solution both bead-like and cell-like particles show the same characteristics, but when the buffer conductivity is below the conductivity of cell cytoplasm, they no longer show the same characteristics as demonstrated in the Fig. 1a and f. An opaque bead-like particle, as shown in Fig. 1a, presents the same electrical characteristics through a