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Detection of pathogens with impedance analysis in a lab on a chip

E.J. van der Wouden[a]a*, M. Super[b], D E. Ingber [b], A. van den Berg[a]

[a]*MESA+ Institute for Nanotechnology, University of Twente ,7500 AE Enschede, the Netherlands*

Biomaterials Evolution Platform, Wyss Institute at Harvard, Boston MA 02115, United States

Abstract

In this work, we demonstrate an impedance approach to detect pathogens bound to magnetic beads. The approach was demonstrated with C-Albicans. We show that when an appropriate frequency is applied to two electrodes present in a microfluidic channel the impedance change in the detection volume can be used to distinguish between unbound beads and beads bound to pathogens. Furthermore, we present a model to relate measured impedance changes to an effective particle diameter.

Keywords: Impedance analyses; lab on a chip; sepsis; magnetic beads

1. Introduction

C-Albicans are fungi belonging to the yeast family and are a known cause of infections like sepsis. In the case of sepsis the early detection and characterization of pathogens present in low concentrations is crucial. Cells present in a solution can be concentrated magnetically by selectively binding them to magnetic beads, after which subsequent analyses of the concentrated sample can be performed. We use 1 μm MyOne Dynabeads magnetic particles coated with the opsonin protein; Mannose Binding Lectin

* E.J. van der wouden. Tel.: +31(0)53 4893944; fax: +31(0)53 4895653.
E-mail address: e.j.vanderwouden@utwente.nl.

(MBL); MBL is a key component of the innate immune system, which binds to carbohydrate structures containing mannose, N-acetyl glucosamine and fucose on the surface of pathogens, and that are not found on mammalian cells[1]. The Albicans have a high mannose sugar and the MBL–coated beads bind strongly to this yeast in the presence of calcium. Here we test if magnetic beads can be distinguished from beads bound to C Albicans in a microfluidic channel with patterned electrodes (Figure 1) via impedance measurements in a detection volume of 23 picoliter, an approach similar to previous research in our group[2]. Microfluidic channels are etched in glass, with a depth of 19 µm and width in the detection region of 40 µm. Planner 100 nm high Ti/Pt electrodes are 20 µm in width and 30 µm separated. A frequency characterization is shown in figure 2 of a fabricated device filled with PBS buffer, three different regimes can be distinguished; At low frequencies the electrical double layer, a thin layer of charge accumulated at the electrodes surfaces, dominates the frequency behaviour. At high frequencies parasitic capacitances of lead wires and that of electrode cell dominate. In the intermediate region the resistance of the electrolyte resistance dominates the impedance from approximately 30 kHz up to 1MHz, in this regime cells or beads passing the electrodes can be detected [3].

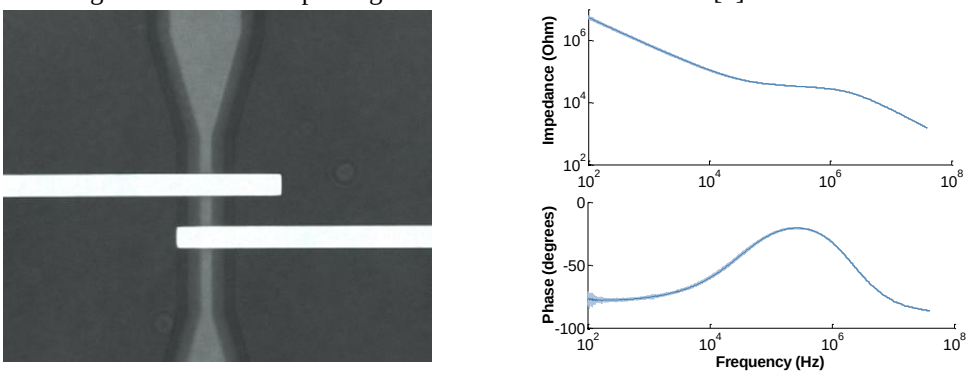
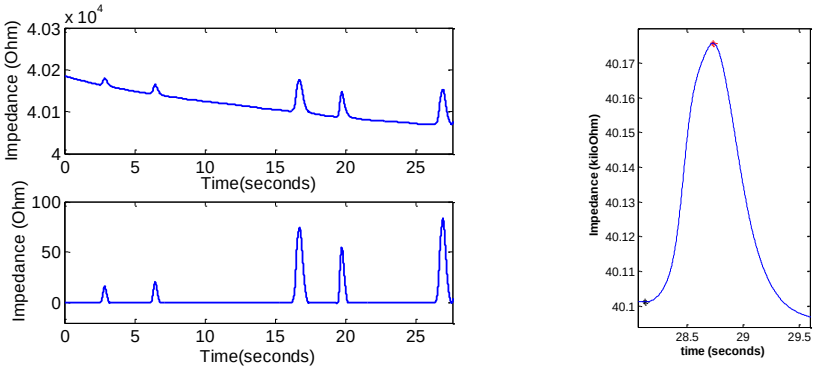


Fig. 1. (left): micrograph image of a chip in the detection region..; (right) Gain phase characteristic. Top shows the Impedance, bottom phase as a function of frequency.

Figure 2a shows measurements with C Albicans the figure shows the raw signal and the corrected peaks after signal processing to correct for drift in the measurement. Impedance detection was performed in PBS buffer and 5 mM $CaCl_2$ with a concentration of both beads and cells of $1*10^6$/ml



(a) (b)

Fig. 2 (a) Electrical detection of cells in PBS buffer passing the detection volume. Top; raw measurement signal, bottom signal corrected for drift.(b) magnification of a measured impedance response for a passing yeast cell.

2. Tortuosity model

To extract size information from the measured impedance change a tortuosity model was used. When cells are present in the detection region the resistance increases due to two factors; firstly, the effective conducting cross-section reduces. Secondly, field lines have to travel around the cell which leads to an increase in the apparent length, also known as tortuosity. Formula 1 gives the relative conductivity change; γ, as a function of the volume fraction of cells (ε) and the tortuosity (ξ) which is equivalent to the ratio in effective cross-section and length with and without a cell present. This in turn can be expressed as the measured ratio in resistance with and without a cell in the detection volume. In formula one R, $R_{\varepsilon,}$ A, $A_{\varepsilon,}$ L, L_{ε} are respectively the resistance, cross-section and length without and with a particle present in the detection volume

$$\gamma = \frac{A_{\varepsilon} / A}{L_{\varepsilon} / L} = \frac{R}{R_{\varepsilon}} = \frac{1-\varepsilon}{\xi} \tag{1}$$

$$\xi = 1 + 0.55\varepsilon \tag{2}$$

For low volume fractions (0-20%) the tortuosity as a function of the volume fraction is given by equation 2; equation 3 can now be used to relate the relative impedance change to a volume fraction of the non-conducting spherical particle[4].

$$\varepsilon = \frac{-20 \cdot (\gamma - 1)}{11\gamma + 20} \tag{3}$$

When the volume fraction calculated with equation 3 is multiplied with the total detection volume of 23 picoliter the cell volume is known and with this also the diameter. Figure 3a gives the diameter distribution for 369 peaks measured with PBS buffer with C Albicans yeast cells and 1 µm MyOne magnetic beads coated with MBL. A normal distribution fit was applied to the populations with sizes larger and smaller than 1.5 µm. The calculated average size for the C-Albicans was 4.3 µm with a standard deviation of 0.91, the average of the MyOne Dynabeads 1.05 µm with a standard deviation of 0.2 µm. Figure 3a shows 2 clearly separated populations, although the distribution for magnetic beads is slightly overlapping with the population of beads bound to C Albicans, possibly due to clustering of magnetic particles over time. For future measurements this issue could be solved by incorporating an additional measurement frequency, above 1 MHz were cells are no longer insulating.

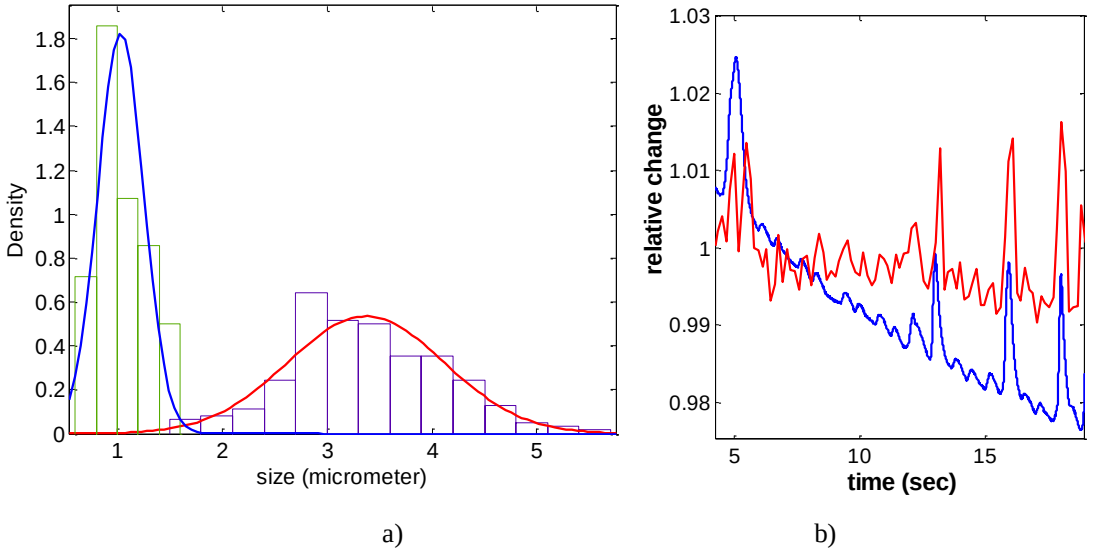


Fig.3: left) Effective diameter extracted from measured impedance changes, with a normalized fit for populations smaller and larger than 1.5 µm. Right) Impedance (blue) and optical signal (red) of beads and pathogens passing the detection volume.

To verify the detection principle optically, the impedance change and optical signal were measured simultaneously in the detection volume as shown in figure 3 b. To increase the optical signal GFP expressing yeast cells were used. In the figure a clear distinction can be made between smaller peaks from the beads and the larger impedance change of the passing cells, indicating the potential of this method for pathogen detection.

3. Conclusions

An impedance approach was developed and tested to distinguish pathogens bound to magnetic beads from unbound beads. This approach allows concentrating prior to analyses so that pathogens in low concentrations can be detected with sufficient reliability. Furthermore, a simple tortuosity model was forwarded with which measured impedance changes can be related to an effective particle diameter.

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