A MONOLITHIC DIELECTROPHORETIC CHIP FOR REAL-TIME LOW-ABUNDANCE BACTERIA DETECTION

*Kruthika Kikkeri and Masoud Agah**
VT MEMS Lab, Bradley Department of Electrical & Computer Engineering, Virginia Tech, USA

ABSTRACT
This paper reports, for the first time, the development of a low-cost, disposable, monolithic chip integrating bacteria enrichment using passivated-electrode insulator-based dielectrophoresis (πDEP) and capacitively-coupled bioimpedance sensing. This integrated chip enables real-time detection of small populations of bacteria in water. The platform operates by selectively concentrating bacteria through DEP forces produced by tunable AC electric fields. Following enrichment and release, bioimpedance measurements are taken concurrently at multiple frequencies in a focusing region. Bacteria detection capabilities of the chip is demonstrated by testing *Staphylococcus epidermidis* at various concentrations and enrichment times. For the current application, the most sensitive operation frequency is determined to be 100 kHz. The chip successfully detects as few as hundreds of bacteria allowing to be utilized for pathogen detection in water samples.

INTRODUCTION
Within the United States, it has been estimated that 7.1 million suffer from microbial infection resulting in nearly 12,000 mortalities every year [1, 2]. Cost effective detection of microbial pathogens is imperative to ensure a safe, contamination-free water supply. Currently, the gold standard method to detect bacteria levels in water is polymerase chain reaction (PCR). In order to achieve the required sensitivity, PCR systems typically utilize laborious and time consuming techniques [3]. As a result, alternative methods to PCR are necessary for rapid, low-cost bacteria detection for low abundance populations. Here, we present a possible alternative to PCR through a microfluidic chip which combines πDEP and bioimpedance measurements.

Our unique πDEP design generates capacitively coupled AC electric fields within the microchannel. The πDEP chip utilizes the concept of positive DEP, as particles in the channel when subjected to the AC electric field move towards areas of high electric field gradient within the microchannel. This isolates the particles to a certain region of the device allowing them to be collected and enriched. The unique πDEP design prevents a direct contact between electrodes and samples preventing common issues associated with electrode-based DEP as well as traditional insulator-based DEP techniques such as fouling, bubble formation, and cross contamination.

Additionally, impedance detection has been shown to reliably and inexpensively detect bacteria [4]. Impedance detection utilizes differences in the electrical properties between particles and suspending medium, which results in changes in the effective complex conductivity of the solution [4, 5]. Thus, by combing the two techniques, we are able to electrically enrich and detect particles in a highly sensitive assay.

Our πDEP platform has previously been used to selectively enrich live bacteria in a live/dead sample [6] using frequency and magnitude tuning of AC electric fields. Moreover, bioimpedance sensing (BIS) has been used to detect bacteria populations in water with limited sensitivity [7, 8]. Though promising, the low detection limit of relying of BIS make it unsuitable for environments with low-abundance levels of bacteria. The combination of DEP and BIS into single low-cost chips allows us to create distributed sensor nodes at high risk points and to continuously monitor and characterize the bacteria presence in real samples almost at the onset of the bacteria/biofilm formation.

DIELECTROPHORESIS THEORY
DEP is the motion of polarizable particles that are suspended in an electrolyte when subjected to a spatially non-uniform electric field [9]. The DEP force experienced by a spherical particle suspended in a medium is:

$$\mathbf{F}_{DEP} = 2\pi R^3 \varepsilon_m Re\left[f_{CM}\right]\nabla|\mathbf{E}|^2 \quad (1)$$

where R is the radius of the particle, ε_m is the permittivity of the medium, $\mathbf{E}$ is the local electric field. $Re[f_{cm}]$ is the real part of the Clausius-Mossotti factor which is:

$$f_{CM} = \left(\varepsilon_p^* - \varepsilon_m^*\right)/\left(\varepsilon_p^* + 2\varepsilon_m^*\right) \quad (2)$$

where ε_p^* and ε_m^* are the complex permittivities of the particle and the medium, respectively [9, 10]. A thorough modeling and analysis of DEP can be found in [10]. From Equation 2, we can see that the properties of particle, such as size and membrane and cytoplasm properties cause cells to experience unique DEP forces for a given electric field gradient. Additionally, we can see that the properties of the particle in comparison to the medium greatly impacts the movement of the particle under the DEP forces. Positive DEP is when the particle conductivity is greater than the medium it is suspended in, causing the particle to move towards areas of high electric field gradients.

Particle movement can be determined through the summation of the forces acting upon the particle. The DEP mobility μ_{DEP}:

$$\mu_{DEP} = \frac{\epsilon_\eta r^2}{3\eta} Re\left\{\frac{\tilde{\sigma_p}-\tilde{\sigma_m}}{\tilde{\sigma_p}+2\tilde{\sigma_m}}\right\} \quad (3)$$

where η is the viscosity of the medium [10]. The mobility can be determined by summing the DEP force and the frictional force term due to drag as expressed by Stoke's equation:

$$f = 6r\pi\eta v \quad (4)$$

Thus, to observe trapping, the DEP force must overcome the electrokinetic and hydrodynamic velocities of the medium. Dielectrophoresis chips can be designed to selectively trap particles by tuning the electric field gradients by channel geometry or electric field magnitude and frequency.

EXPERIMENTAL METHODS

Chip Fabrication

A simplified version of the πDEP fabrication process was used for the sensing system [5]. The monolithic chip fabrication process is depicted in Figure 1 which consists of two compartments: a disposable microfluidic component and a reusable transducer component. The disposable microfluidic component consists of an array of closely-spaced pillars to generate high-electric field gradients and a narrow channel to focus the passage of enriched bacteria following their release. The disposable component is fabricated in polydimethylsiloxane (PDMS) using a dry etched silicon negative master wafer, shown in Figure 1A-D. The solidified PDMS is then bonded to a 100µm thick cover glass slide.

The reusable (detachable) transducer component, fabricated on a glass substrate, consists of an array of chrome/gold electrodes for AC excitation to generate DEP forces on bacteria around pillars and for impedance sensing along the focusing channel. Electrodes were fabricated through photolithography and deposition of chrome/gold. The two components are placed on top of each other with minimal alignment and attached using tape.

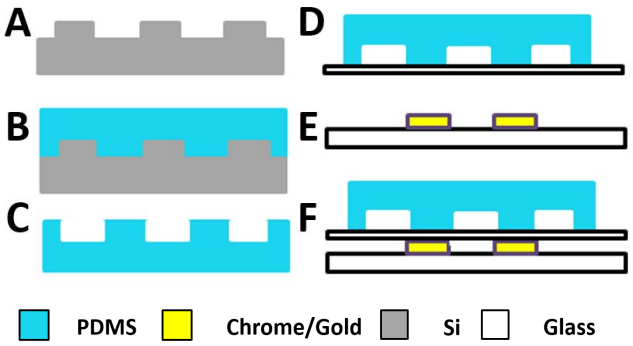

Figure 1: (A) Lithography to pattern the Si wafer (B) Pour liquid PDMS onto the Si master and cure at 100˚C for 40 min (C) Remove cured PDMS from wafer and punch ports (D) Bond to glass slide using oxygen plasma (E) Deposit electrodes on separate glass slide (F) Align and attach the two parts. Note that (D) is the disposable microfluidic component and (E) is the reusable transducer component of the monolithic chip.

Figure 2 shows an optical image of the monolithic chip with close-ups of the DEP and BIS regions. The insulating pillars are 100 µm in diameter with 50 µm spacing. The focusing channel is 20 µm wide and 400 µm long. The entire channel is 40 µm deep. Both the DEP and BIS electrodes are spaced 1000 µm apart. The overall size of the entire chip is around 3 cm × 2.5 cm.

One pair of electrodes was connected to a 50 MHz function generator (B&K Precision, CA, USA) and a

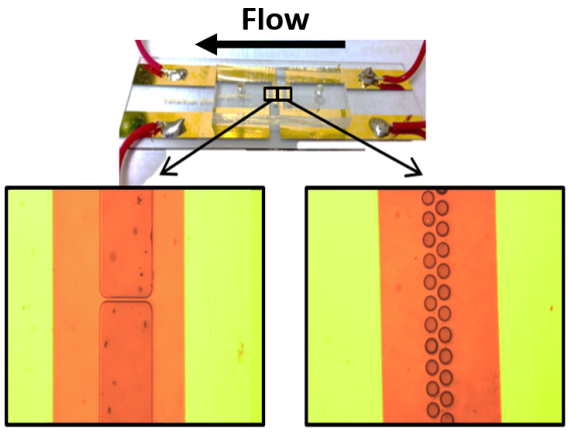

Figure 2: Top view and close up of fabricated device, where the DEP posts are on the right and the BIS channel is on the left.

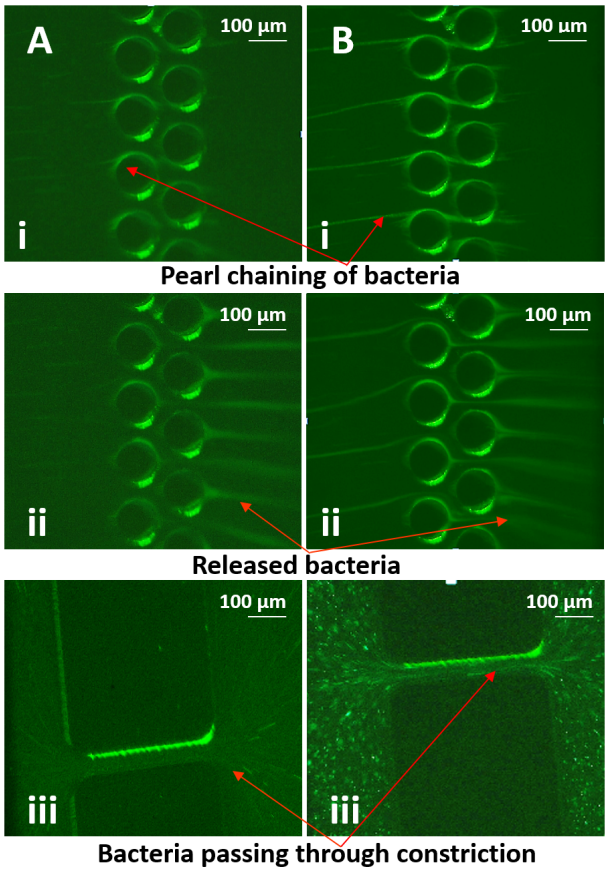

Figure 3: Images of (A) 60 and (B) 600 second DEP enrichment when (i) an AC electric field is applied at the optimized frequency and voltage of 400 kHz and 300 Vpp, causing bacteria to move towards the high electric field regions (around the pillars) and "trap," pearl chaining is observed (ii) bacteria are released as a result of turning off the electric field (iii) BIS measurements are taken around the focusing channel.

voltage amplifier (FLC Electronics AB, Sweden) to initiate DEP forces in the DEP region. Another pair of electrodes was connected to an impedance spectroscope. These electrodes surround the BIS region for impedance sensing.

Chip Operation

Various *Staphylococcus epidermidis* concentrations were subjected to DEP forces induced by the application of the AC electric field causing trapping around the pillars as seen in Figure 3A-Bi. Enriched bacteria were then released by turning off the signal as shown in Figure 3A-Bii.

The enriched cells then flowed through the narrow BIS region (Figure 3A-Biii) where impedance measurements were recorded. Additionally, following the removal of the electric field, the signal was almost immediately turned back on. This ensured that only the enriched sample was measured.

Operation parameters for the AC signal and impedance measurements were optimized. By sweeping the voltage and frequency of the electric field, it was found that 300 Vpp, 400 kHz produced a trapping efficiency close to 100% as seen in Figure 3A-Bi. Additionally, impedance measurements were taken at various frequencies. 100 kHz was found to be the most sensitive frequency for impedance sensing.

It is important to note that using a chip without the narrow channel, bacterial enrichment alone failed to produce any measurable changes in BIS, even for high concentrated samples. This clearly highlights the necessity of the innovative focusing region for bacteria detection even under extremely low concentrations.

RESULTS AND DISCUSSION

Sensitivity of the device was determined by varying the concentration of the *Staphylococcus epidermidis* cell solutions at 10^2, 10^4, 10^6, and 10^8 cells/mL. Enrichment time was varied as 30, 60, 180, 300, 480 and 600 seconds. Figure 3A-B shows the trial for 60s and 600s DEP enrichment, respectively. It shows how the longer trapping times result in an increase in the number of collected cells.

Figures 4 and 5 display impedance measurements determined at varying enrichment times. The solution concentration was constant at 10^8 cells/mL. The baseline impedance was 3.1 x10^4 Ω. This value was a combination

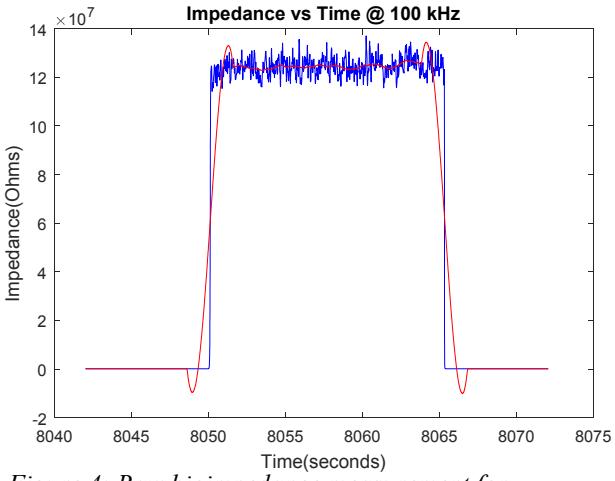

Figure 4: Raw bioimpedance measurement for Staphylococcus epidermidis concentration of 10^8 cells/mL (blue) with smoothed curve (red) for 60 seconds DEP trapping.

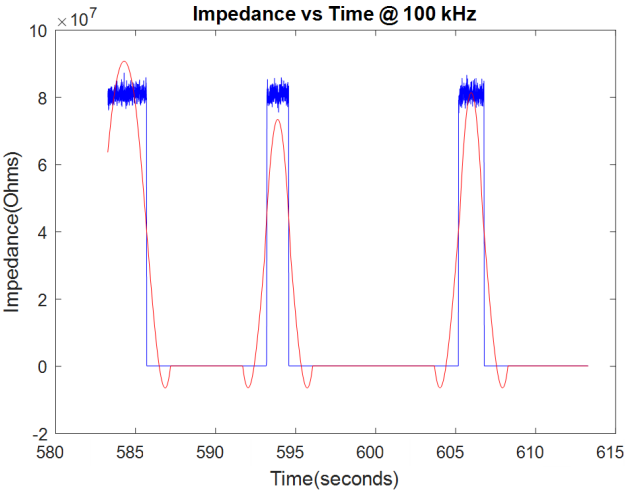

Figure 5: Raw bioimpedance measurement for Staphylococcus epidermidis concentration of 10^8cells/mL (blue) with smoothed curve (red) for 600 seconds DEP trapping.

of noise and the impedance value of the deionized water medium. Following removal of the electric field, the release of the bacteria cells generated a step increase in the impedance output as seen in Figures 4 and 5. The raw data (blue) shown in Figures 4 and 5, was found to have some fluctuation. To analyze the raw data, a filtering algorithm was utilized to smooth the curve (red in Figures 4 and 5).

When enriched for 60 seconds, the cell solution had an average peak impedance value of about 8.4×10^8 Ω. In contrast, 600 second enrichment had an average peak of 12.6×10^8 Ω. Additionally, it was observed that for 60 second enrichment, the duration of the peak was on average 2 seconds, while it was 15 seconds when cells were enriched for 600 seconds. Therefore, the area of the step increase was calculated to account for the variations in both the average impedance and the duration of the peak.

The area under the step increase for each enrichment time was plotted on Figure 6.

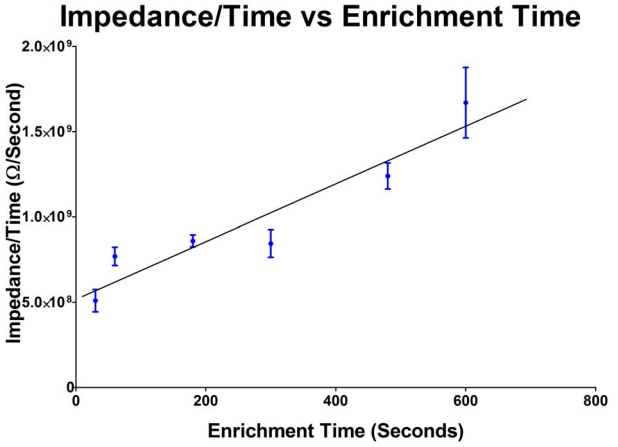

Figure 6: Area under the BIS peaks for each DEP enrichment time point for 10^8 cells/mL of Staphylococcus epidermidis with an R-square value of 0.9483.

It can be seen that unique impedance per second values were obtained for each enrichment time. Thus, it is observed that longer enrichment times resulted in larger step increases for longer durations. The best fit line was also plotted on Figure 6 and found to have an R-squared value of 0.9483. This shows there is a linear trend between enrichment time and the output impedance measurement. The linear tread also enables us to not only detect these microbes in the solution but also quantify the amount of bacteria based on the step increase.

A similar trend was found when the bacteria concentration was varied. Figure 7 depicts the area under the step increase as a function of the initial bacteria concentration for a constant enrichment time of 300 seconds. The line of best fit for these trials had an R-squared value of 0.9285.

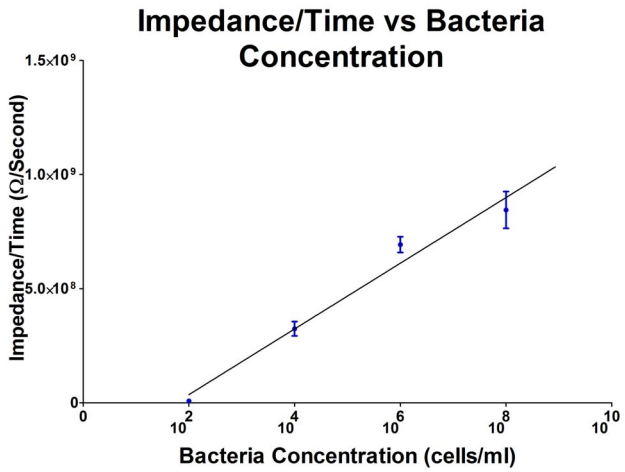

Figure 7: Area under the BIS peaks for each bacterial cell concentration for 300 second enrichment of Staphylococcus epidermidis with an R-square value of 0.9285.

Based on the flow rate, bacteria concentration and enrichment times, it was determined that the lowest number of bacterial cells that produced unique impedance values was in the hundreds range. The number of bacteria cells passing through the channel was confirmed with videos of DEP and BIS region during chip operation.

CONCLUSION

We fabricated a DEP monolithic chip capable of detecting low abundance populations of bacteria in water solutions. We have demonstrated that the device is sensitive enough to provide BIS measurements for as few as hundreds of bacterial cells. This low-cost, reusable chip allows for bacteria detection in a simple assay. Additionally, the chip features electric field tuning which translates to selective enrichment of the bacterial sample, ensuring that only desired particles are detected.

As this system is primarily a proof-of-concept, further modification and testing will be required to optimize the design. The flexibility of the DEP design enables further modification of the channel and electrode geometry to improve detection sensitivity. Regardless, the current design is highly promising due to its high sensitivity and relatively fast measurements compared to PCR testing. We conclude that this microchip has the potential to be utilized as a rapid detection microsystem for low-abundant bacterial populations.

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CONTACT

*M. Agah, tel: +1-540-231- 2653; agah@vt.edu