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Electro-Disruption of *Escherichia coli* Bacterial Cells on a Microfabricated Chip

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Abstract— **A miniaturized system for sample preparation of relevant bacterial pathogens has been developed using a variety of microfabrication techniques. The system manipulates and disrupts *Eschericha coli* bacterial cells using dielectrophoresis, electroporation and enzymes. The microchip consisted of circular gold electrodes patterned on glass using standard photolithography housed in a PDMS chamber. The bacterial lysis efficiency by electroporation and enzymatic degradation was evaluated on the microchip. The miniaturized system was capable of concentrating and aligning bacterial cells in regions of high electric field by dielectrophoresis. The miniaturized sample preparation system had a lysis efficiency of 17 % when the bacterial cells were suspended in a 0.25 M sucrose solution and increased to 80 % when the bacterial cells were suspended in a solution containing 0.25 M sucrose and 10 KU/ml lysozyme. Sample preparation is a limiting factor for the successful application of molecular pathogen detection methods. Therefore, the development of miniaturized systems useful for sample preparation will improve molecular detection methods of bacterial pathogens.**

I. Introduction

Infectious diseases produced by different types of bacterial pathogens pose an ongoing risk to human health. It has been recognized that the rapid detection of pathogens would improve patient treatment and outbreak management [1]. The development of molecular methods for the detection of bacterial pathogens has helped to produce tests which are faster and have more sensitivity and specificity than conventional culture-based methods [2]. However, the sample preparation stage prior to detection remains the limiting factor for the successful application of molecular diagnosis of relevant pathogens [3]. Some of the persisting problems associated with sample preparation are: lack of standardization, long turn around time and high complexity. The development of reliable sample preparation systems is indispensable for the successful application of molecular diagnosis of pathogens. Unlike conventional sample preparation methods, researchers applying Lab-on-a-chip (LOC) technologies are developing a more diverse set of strategies to suitable extract target nucleic acids or other molecules from biological samples. Some of the strategies, such as the use of enzymes for pathogen lysis [4] or silica microparticles for nucleic acid extraction [5], have been borrowed from conventional methods whereas other strategies have been designed using the unique capabilities of LOC technology [5]. The main LOC methods currently under investigation for nucleic acid extraction on miniaturized platforms are based on electrical [4, 6, 7], mechanical [8-10] and chemical methods [11, 12].

The use of electric fields to develop miniaturized sample preparation systems has been explored with promising results. Nonuniform A.C. electric fields have been employed in dielectrophoresis (DEP) to concentrate and align bacterial pathogens in regions of high electric fields, and pulsed electric fields have been employed in electroporation to electro-disrupt the bacterial cells in order to release their nucleic acid to the medium for subsequent extraction. The extracted nucleic acids can be used for downstream amplification and detection of pathogens [4, 6, 7].

Despite the demonstration of its feasibility, very few studies have evaluated the specific effect of electric field pulses and enzymes on the bacterial lysis efficiency of the microchips.

The purpose of this study was to develop a LOC-based system useful for the manipulation and disruption of *E. coli* bacterial cells using DEP, electroporation and enzymes. This system has the capability to be used for sample preparation of bacterial pathogens. The present work investigated: (1) the pre-concentration of bacterial cells by positive dielectrophoresis, and (2) the bacterial cell lysis efficiency by electroporation and enzymatic degradation.

II. Materials and Methods

The organisms used for this study were an ATCC strain of *E. coli*. The organisms were cultured overnight in Tryptic Soy Agar (BD, lot 5081624) at 37 ^{o}C for 12 hours before experimentation.

The microchips utilized in this investigation were fabricated using standard photolithography [13]. The microelectrodes were made of a 220 nm layer of Chromium/Gold (20 nm/200 nm) patterned on glass. As shown in figure 1, the microchip consisted of a 5 mm × 5 mm array of circular microelectrodes. The individual microelectrodes were spaced 10 μm apart.

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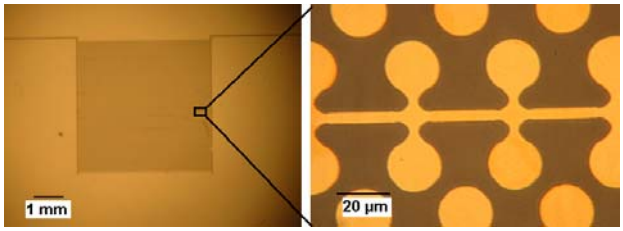

Fig. 1. Shape and layout of the circular microelectrodes patterned on glass. The individual microelectrodes were 20 µm in diameter and spaced 10 µm apart.

The entire microchip was coated with a layer of negative photoresist SU-8 2000.5 and a layer of negative photoresist HPR-504. The function of the coating was to isolate the microelectrodes from the liquid sample in order to avoid bubbles or electrolysis during experimentation. The microchip was spin-coated (Brewer Science Cee 100CB Coat-Bake System) with negative photoresist SU-8 2000.5 (Microchem, Lot 05090707) at 500 RPM for 15 seconds and 2000 RPM for 45 seconds. Then, a second coating of negative photoresist HPR-504 at 500 RPM for 10 seconds and 4000 rpm for 40 seconds was applied, forming a layer thickness of 1.4 µm over the electrodes. After the application of each coating, the microchip was dried on a hot plate (Brewer Science Cee 100CB Coat-Bake System) at 110 ^{o}C for 20 minutes to harden the coatings and to remove any residual solvent.

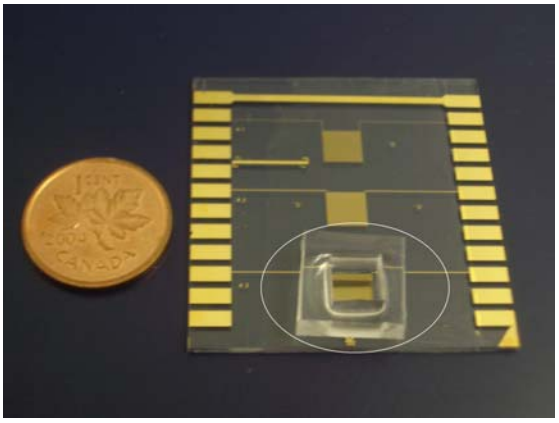
Fig. 2. PDMS chamber used to hold the sample on the microchip.

A chamber used to contain the sample on the microchip was fabricated using polydimethylsiloxane (PDMS. Sylgard, lot 2144245). Figure 2 shows the chamber placed on the microchip. The overall dimensions of the chamber were 10 mm length × 10 mm width × 5 mm height, housing a square well of dimensions 5 mm × 5 mm × 5 mm in the middle of the PDMS piece.

For the experimental set up, the PDMS chamber was placed and secured on top of the 5 mm × 5 mm microchip. The microchip was mounted on a microscope stage (Zeiss, Model Axiovert 25) and connected to a wide band signal generator (BTX, Model ECM 2001) using a specially designed printed circuit board. A 100-µl aliquot of a solution containing 100 µg/l of bovine serum albumin (BSA) was added to the chamber and left there for 20 minutes to coat the chamber walls (EMD lot 1855B47). The BSA formed a hydrophobic layer on the microchip used to minimize the adsorption of cell debris and other biomolecules onto the microchip surfaces.

Once the experimental set up was ready, bacterial colonies were scraped from the agar plate using a culturing loop and placed in a 1-ml solution containing 0.25 M sucrose (EMD, lot 855B05). Using a vortrex (VWR model MV1), the solution with the bacteria was mixed for 5 seconds and equaled to a 0.5 McFarland Standard (Remel, Lot 204983) using a spectrophotometer (Thermo, Model Spectronic 20D+) at 625 nm and 0.25 M sucrose solution. The McFarland standard was used as a reference to adjust the turbidity of bacterial suspensions so that a bacterial cell concentration was always within a given range. Therefore, this bacterial suspension was called: the "0.5 McFarland standard-equaled bacterial suspension".

The bacterial lysis efficiency of the microchip was determined by two sets of experiments. The first set of experiments used only electroporation as the means of disrupting the bacterial cells, and was conducted in order to test the bacterial lysis efficiency of the microchip when the bacterial cells were in a solution containing only 0.25 mM sucrose. The second set used a combination of electroporation and lysozyme as the means of disrupting the bacterial cells, and was conducted in order to test the bacterial lysis efficiency of the microchip when the bacterial cells were in a solution containing 0.25 M sucrose and 10 KU/ml of lysozyme.

The "0.5 McFarland standard-equaled bacterial suspension" was used as the sample for the set of experiments without lysozyme. For the experiments with lysozyme, enough lysozyme (Sigma, lot 94k1454) was added to the sample to reach a concentration of 10 KU/ml and the sample was incubated at 30 ^{o} C for 10 minutes. Lysozyme is an enzyme known to degrade the cell wall of certain bacterial cells. For each set of experiments, 100 µl of sample was used.

The first set of experiments, where 0.25 M sucrose was the only component used in the bacterial solution, consisted of two tests. The first test was a control test, where no electric field was applied, and the second test was a treatment test where a 90-second electric field at 10 V_{rms} AC was applied in order to attract the bacterial cells to regions of high electric field as a result of positive dielectrophoresis, immediately followed by the application of 360 electric pulses at 300 V DC and 500 µs pulse duration in order to disrupt the bacterial cells by electroporation. The second set of experiments, where the bacterial cells were in a solution containing 0.25 M sucrose and 10 KU/ml of lysozyme, also consisted of two tests. The first test was also a control test, where no electric field was applied, and the second test was a treatment test where a 90-seconds electric field at 10 V_{rms}

AC was applied immediately followed by the application of 360 pulses at 300 V DC and 500 µs pulse duration. Three replicates of each control and treatment test were conducted.

After each treatment, the sample was recovered from the microchip using a micropipette (Eppendorf, model reference) and placed in a 1.5-ml test tube containing 900 µl of 0.25 M sucrose solution. Serial dilutions of this sample were cultured in Tryptic Soy Agar for 24 hours in order to determine the bacterial cell concentration present in the recovered sample. The lysis efficiency was estimated by determining the difference between the bacterial cell concentration of the control tests and the bacterial cell concentration of the treatment tests.

III. RESULTS AND ANALYSIS

The concentration and alignment of *E. coli* bacterial cells in regions of high electric field was successfully demonstrated using dielectrophoresis. Figure 3 shows pictures of *E. coli* bacterial cells placed on the microchip dyed with methylene blue. Figure 3a shows the bacterial cells scattered randomly over the microchip before the application of the electric field. Figure 3b shows the bacterial cells under DEP, concentrated in the regions of high electric field when an electric field at 10 V_{rms} AC and 1 MHz was applied for 90 seconds. Concentration and alignment of bacterial cells in regions of high electric fields serves two purposes: the pre-concentration of pathogens from the sample and the alignment of pathogens on regions of high electric field for subsequent application of electric pulses during electroporation. The concentrating capability of the system is useful for sample preparation of biological specimens where target pathogens are not abundant.

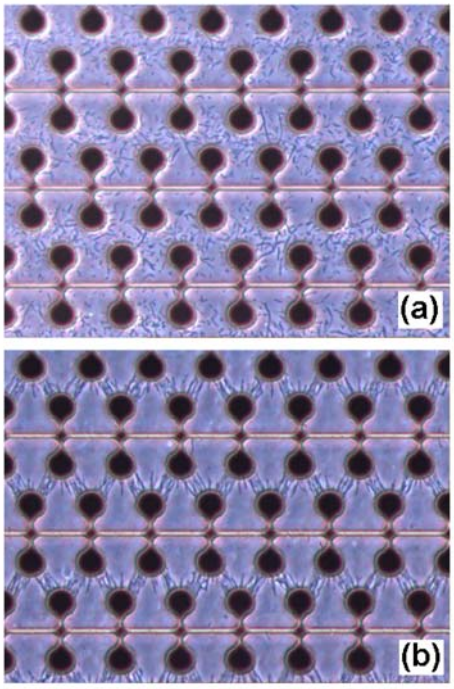

Fig. 3. Concentration and alignment of *E. coli* bacterial cells dyed with methylene blue in regions of high electric field by positive dielectrophoresis. The field was applied with a voltage of 10 V_{rms} and a frequency of 1 MHz. The conductivity of the media was 6 µS/cm.

Immediately after the bacterial cells were aligned in the regions of high electric field by DEP, the high voltage electric pulses were applied in order to disrupt the cells by electroporation. Figure 4 shows the bacterial cell debris after the electroporation treatment, demonstrating the disruption of the bacterial cells by electroporation. Bacterial cell debris can be observed in the locations subjected to a high electric field during the treatment.

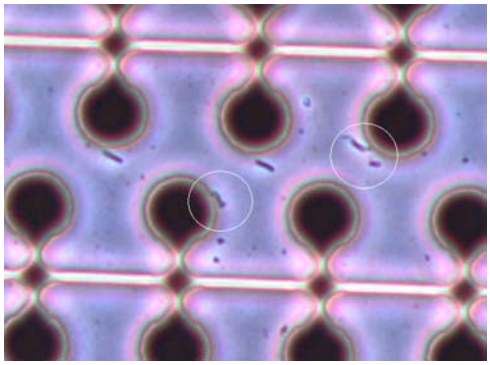

Fig. 4. The white circles show some of the cell debris present after the electroporation treatment.

The bacterial lysis efficiency by elctroporation was enhanced when 10 KU/ml of lysozyme was used in the solution containing the bacterial pathogens. Figure 5a shows the results of two sample groups treated without lysozyme. In both groups the bacterial cells were in a 0.25 M sucrose solution. The control group, which was not subjected to an externally applied field, had an average bacterial concentration of 1.2×10^6 cells/ml. The treatment group, which was treated with 360 electrical pulses at 300 V DC and 500 µs pulse duration, had an average bacterial concentration of 1×10^6 cells/ml. This represented a reduction in bacterial concentration of 17 %. Figure 5b shows the results of two other sample groups treated with lysozyme. In both groups the bacterial cells were in a solution containing 0.25 M sucrose and 10 KU/ml of lysozyme. The control group, which was not subjected to any electric pulses, had an average bacterial concentration of 8.3×10^5 cells/ml. The treatment group, which was treated with 360 pulses at 300 V DC and 500 µs pulse duration, had an average bacterial concentration of 1.6×10^5 cells/ml. This represented a reduction in bacterial concentration of 80.7 %. The enhancement of lysis efficiency by adding lysozyme prior to the electroporation protocol was due to the fact that the enzyme lysozyme degraded the bacterial cell wall. The degradation of the bacterial cell wall, which acts as a barrier to protect the cell membrane, facilitated the creation of pores in the membrane leading to the disruption of the bacterial

cell membrane. The combination of electroporation and bacterial cell wall-degrading enzymes improved the lysis efficiency of the system. It was furthermore observed that the lysozyme treatment alone had an impact on the bacterial cell lysis. This effect can be observed on the control groups of figures 5a and 5b, which were not treated with any electric pulses. The control group on figure 5a, which had not lysozyme added, had a bacterial concentration of 1.2×10^6 cells/ml whereas the control group on figure 5b, which had lysozyme, had a bacterial concentration of 8.3×10^5 cells/ml. Other authors have also used different enzymes such as proteinase K in combination with electroporation [4]. However, the specific effect of enzymes on bacterial lysis by electroporation had not been fully investigated before.

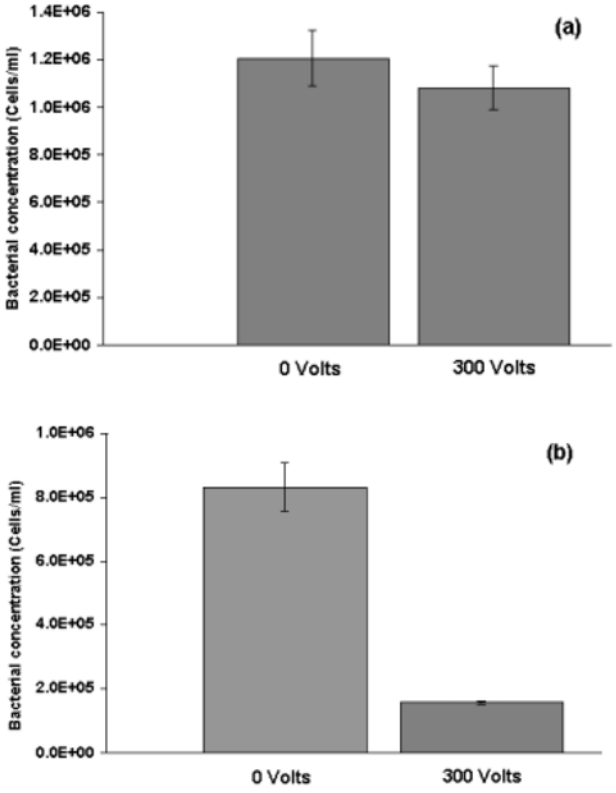

Fig. 5. The combined use of electroporation and lysozyme improved the bacterial cell lysis efficiency. (a) Shows the reduction of 17 % in bacterial cell concentration when the cells where in a solution containing only 0.25 M sucrose, and (b) shows a reduction of 80.7 % in bacterial cell concentration when the cells where in a solution containing 0.25 M sucrose and 10 KU/ml of lysozyme.

IV. CONCLUSIONS

A miniaturized system for the manipulation and disruption of *E. coli* bacterial cells using dielectrophoresis, electroporation and lysozyme was developed using standard microfabrication methods. The system is capable of concentrating and aligning the bacterial cells in regions of high electric field employing positive DEP, and disrupting the bacterial cells by electroporation. This system can be used as part of miniaturized sample preparation system for relevant bacterial pathogens.

The miniaturized system was capable of concentrating and aligning the bacterial cells in regions of high electric field by DEP. The cell concentrating capability of the system is useful for sample preparation of specimens containing small amounts of the target pathogen that needs to be identified.

The combination of electroporation and lysozyme improved the bacterial cell lysis efficiency of the system. The miniaturized sample preparation system had a lysis efficiency of 17 % when the bacterial cells were suspended in a 0.25 M sucrose solution. The lysis efficiency increased to 80 % when the bacterial cells were suspended in a solution containing 0.25 M sucrose and 10 KU/ml lysosyme.

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