A MICROFLUIDIC SAMPLE PREPARATION DEVICE FOR PRE-CONCENTRATION AND CELL LYSIS USING A NaNOPOROUS MEMBRANE

Md. Shehadul Islam 1, Kacper Kuryllo 2, P. Ravi Selvaganapathy 1*, Yingfu Li 3 and M. Jamal Deen 3
1 Department of Mechanical Engineering, McMaster University, ON, Canada
2 Department of Biochemistry and Biomedical Sciences, McMaster University, ON, Canada
3 Department of Electrical Engineering, McMaster University, ON, Canada

ABSTRACT
Pathogenic bacteria (E. coli-O157, Salmonella) are the main reasons for waterborne illness. Lysis of these bacteria is an important step in molecular identification of these pathogens. Electrical method has been chosen here as it is reagent free and hence more conducive automated sample preparation. We use commercially available nanoporous membranes sandwiched between two microfluidic channels to create thousands of parallel nanopore traps for bacteria and lyse them. Accumulation and lysis of bacteria on the nanoporous membrane is demonstrated by using Bacterial Viability Kit. The efficiency of the device was determined through plate counting and was 90% at applied potential of 300 V.

KEYWORDS: Cell lysis, Electroporation, Microfluidics, Escherichia coli, nanopores

INTRODUCTION
Microbial contamination of water is the major reason for prevalence of waterborne diseases. Conventional cell culture based methods for detecting this contamination are time consuming and compromise the timeliness of health advisory warnings [1]. Molecular biology-based approaches such as the use of D A assays to analyze the presence of pathogens in water sample are accurate, extremely sensitive and can be performed rapidly [2]. In these assays, D A and other biomarkers that are present inside the cell have to be extracted from within by breaking down the cell membrane. This process, known as cell lysis, is an important step in sample pre-treatment for D A based analysis of water samples.

Conventionally, in the laboratory, chemical and mechanical methods are used to lyse the cell [3]. Recently, a number of microfluidic devices have been developed that lyse cells using chemical, thermal, mechanical, electrical and electrochemical methods [4-5]. Compared to the other methods, electrical lysis is more suited for online and automated sample preparation as it is rapid and reagent free. The working principle for electrical cell lysis is similar to electroporation. Electroporation was originally developed in the early 1980s by Eberhard cumann et al. [6] as a method to temporarily permeabilize the cell membrane and form pores through which external genetic material could be inserted into the cells. If the duration of the electric pulse is short, the membrane reforms and the cell remains viable. Longer pulses or DC voltages can be applied to irreversibly damage the cell membrane and lyse the mammalian cell without the use of reagents [7]. Both electroporation and electrical lysis require high electric field (critical electric field 1-2 KV/cm [8]). Microfabrication has been used to miniaturize electrodes and place them close to each other, which reduces the voltage required to obtain the critical electric field for lysis [9]. However, microfabricated electrodes are expensive and require the use of AC and pulse electric fields to avoid bubble generation and clogging [10]. Alternatively, high local electric field has been obtained without miniaturizing electrodes by creating a narrow neck in a microfluidic channel. These designs have been used to demonstrate lysis of bacteria [11] and RBC [12] in a continuous flow through manner with DC applied voltages. Although, promising, they consume significant power, are prone to clogging and do not concentrate the lysed D A into a small volume which is important for environmental applications.

In this paper, we develop a continuous flow device that is capable of pre-concentrating bacteria from a sample electrophoretically into a small volume and lyse it using localized high electric field without the use of microfabricated electrodes or lysis reagents. We show that this device avoids the typical problems associated with electrical cell lysis such as Joule heating and bubble generation due to continuous flow operation and the use of low voltages. The throughput of the device can be increased by either increasing the cross sectional area of the membrane interface or by parallelizing the format to accommodate very large sample sizes.

DEVICE DESIGN
The lysis device (Fig 1) consists of a nanoporous membrane sandwiched between two microchannels with electrodes embedded at the reservoirs of the microchannels. An electric field, applied across the nanoporous membrane through the microchannels, electrophoretically transports the bacterial cells suspended in the sample that is flowed in the main sample channel and accumulates it at the intersection of two channels on the nanoporous membrane. Subsequently, increasing the electric field lysates the cells and leads to extraction of the D A through the nanopores into the microchannel below. This device can be operated in a semi-batch or continuous mode to concentrate a large sample with low cell concentration into a small volume before lysis. Furthermore, since the resistance of the nanopores are larger compared to the microchannels and the membrane is thin, a very high electric field can be obtained locally at the nanopores upon application of a small voltage.
EXPERIMENTAL

Experimental setup (Fig 2) to study the accumulation of bacteria and its lysis consists of four major parts: a microfluidic device, bacteria and buffer handling system (syringe pumps, syringe, and inlet-outlet connection), power system (power supply and electrodes) and monitoring unit (microscope and PC).

To demonstrate accumulation and subsequent lysis of the cell, *E. coli* with bacterial viability kit (Syto 9 and PI) was used. Syto 9 stains viable bacterial cells while propidium iodide (PI) stains nucleic acid that has been released by lysis. Since, these dyes have been used during preparation of sample, intact bacteria fluoresce green (500-550 nm) when excited with blue light (488 nm) and lysed bacteria fluoresce red (595-660 nm) when excited with green light (561 nm).

Initially, the sample channel and the collection channel were loaded with phosphate buffer and platinum wires (diameter 0.3 mm) were inserted into the reservoirs 2 and 6 (Fig 2) and connected with a power source (KEITHLEY 2410). The membrane was first primed by applying 100 V for 5 min. Then all the trapped air bubbles were removed by flowing buffer from reservoir 3 and 5 by syringe. Then sample was injected into the injection channel at a flow rate of 100 μl/hr while buffer was injected through other channel simultaneously at the same flow rate. A syringe was used to fill the buffer in the collection channel. When bacteria were observed to approach the membrane region in the top channel, a potential (50 V) was applied at the electrodes and fluorescent images at the emission frequencies of Syto 9 and PI were recorded. Images were taken every 5 sec to obtain data on cell accumulation. After applying 50 V for three min, the flow in the injection channel and focusing channel was stopped and the flow rate of buffer was increased to 200 μl/hr. This condition was used for 2 min. This operation resulted in the flow of a fixed amount of bacteria (required for characterization) across the membrane region. At the end, the operational voltage was increased to 100-300 V for another 3 min to lyse the accumulated cell population.

RESULTS AND DISCUSSION

**Electrical Lysis:** In this experiment *E. coli* sample (concentration of 10⁶-10⁷ CFU/ml) stained with Syto 9 and PI were introduced in the injection channel and buffer was introduced from focusing channel and sample channel with a flow rate of 100 μl/hr. The bacterial cells were accumulated for 5 min using a procedure similar to the one described above. Subsequently, the flow in the injection channel was stopped and the flow in the sample and the focusing channels were continued in order to flush away the remaining sample solution in the upstream region. Then the applied potential was increased to 300 V which corresponds to local electric field at the nanopores of 1860 V/cm. The intensity of both Syto 9 and PI at the membrane interface during the entire operation is presented in Fig 3 and shows rapid increase in the fluorescent intensity for 3 min after which it plateaus out. Subsequent application of a higher voltage for 300 sec leads to a increase in the intensity of both Syto 9 and PI that also saturates with time. This behaviour is as expected. A continuous flow of the sample tangential to the nanoporous interface for the first 3 min led to the gradual accumulation of bacteria as indicated by the increase in the intensity of Syto 9. Subsequently, when the flow of the sample was stopped and only the buffer flow was maintained in the next 2 min there are no bacteria in the flow to accumulate on the nanoporous interface as indicated by the nearly constant intensity over this period of time. All along the intensity of PI did not increase indicating that while the bacteria were accumulated they did not lyse. Subsequently, when the applied potential was increased to 300 V and applied for 3 min, the Syto 9 fluorescence intensity increased sharply indicating that the cells were lysed. More significantly, the intensity of the PI also increased providing a direct indication that the cells are lysed at this applied potential. The electric field calculated at the nanopores was 1860 V/cm at an applied potential of 300 V which was higher than the threshold for lysis. Lysis was observed in the microchannels as the electric field was around 375 V/cm and well below the threshold.

**Lysis Efficiency:** In order to determine efficiency of lysis at different voltages *E. coli* sample (10⁶-10⁷ bacterial cells/ml) was exposed to applied voltages of 50 V, 100 V, 200 V, and 300 V for a duration of 3 min while flowing at 100 μl/hr. It should be noted that in this configuration, the cells were lysed as they were captured on the membrane. Plate
counting was performed using 10 μl of solution collected from the sample channel and collection channel outlet to
determine the lysis efficiency at various applied voltages. Efficiency was calculated by comparing the plate counts to a
control run where no potential was applied to the device and is shown in Fig 4. They show that there is a threshold below
which there is minimal lysis of the cells. Beyond the threshold, the efficiency of lysis increases with the applied voltage.
It is 56% at 100 V, 82% at 200 V (1232 V/cm) and 90% at 300 V (1860 V/cm) while only 5% were lysed at 50 V. Also,
no colonies were formed from the fluid collected from the collection channel confirming that intact bacteria did not pass
through the membrane.

**CONCLUSION**

In conclusion, we have successfully fabricated a simple and rapid cell lysis system by using low voltage and tested it
for *E. coli* bacteria. The combination of a simple design, easy fabrication and integration of pre-concentration and cell
lysis in a compact form factor makes this an elegant design for sample processing.

**ACKNOWLEDGEMENT**

The research was supported by SERC Strategic Grant and through the Canada Research Chairs Program

**REFERENCES**


**CONTACT**

* Dr. P. Ravi Selvaganapathy, JHE 212B, 1280 Main St W, Hamilton, ON L8S 4L7, Canada; selvaga@mcmaster.ca