SUB-MICROFLUIDIC DEVICES TO OPTIMIZE REMOVAL OF PATHOGENS FROM DRINKING WATER USING SAND FILTRATION

N. Tandogan, Y.A. Zhu, K.T. Wan, and E.D. Goluch*
Northeastern University, USA

ABSTRACT

Sub-microfluidic devices were designed to study bacterial behavior in confinements under fluid flow. This study will provide understanding of the effectiveness of sand filtration, a major water purification strategy in the developing world. *Escherichia coli* and *Staphylococcus aureus* were selected and separately flowed through sub-microscale confinements under different pressure values. Fluorescent images elucidated that strains behave differently in confinements due to distinct cell shape and wall structures, suggesting that transport through similar size confinements is not a simple function of cell size. Other pathogens must be investigated to unravel this phenomenon.

KEYWORDS: Bacteria, Filtration, Confinement, Microfluidic Device

INTRODUCTION

Access to potable drinking water is a growing global issue. Among many filtration processes, slow-sand filtration is an economical alternative for resource-poor areas that is endorsed by the World Health Organization. However, filtration of pathogens using this approach has not been thoroughly examined [1]. The results of our experiments will identify the optimal conditions at which pathogens are removed from drinking water. Microfluidic studies so far have focused primarily on bacterial growth rather than bacterial behavior in these confinements. We propose the use of sub-microfluidic confinement to emulate the porous structure of sand to identify the best type and size of sand as well as the optimal pressure head to apply to the water as it flows through the sand to remove microbes. Schematics of the design can be seen in Figure 1. Since types of soils and common pathogens vary regionally, compiling sets of parameters is crucial for reliably implementing these systems. Fluorescently labeled *Escherichia coli* and *Staphylococcus aureus* were selected as initial model organisms for their different morphology and presence as contaminants in drinking water [2]. Previous studies indicate that bacteria translocate structures that are smaller than their own diameter without externally applied pressure [3,4]. Applying pressure further enhances bacterial transport through these sub-micron structures [5-7]. The cell wall flexibility of bacteria seems to have a pivotal effect on their movement through these structures. A recent study showed that movement of bacteria was significantly altered in tapered channels depending on their cell wall structure [8]. In this study, we investigate the relationship between applied pressure and bacterial morphology on bacterial behavior in confinements.

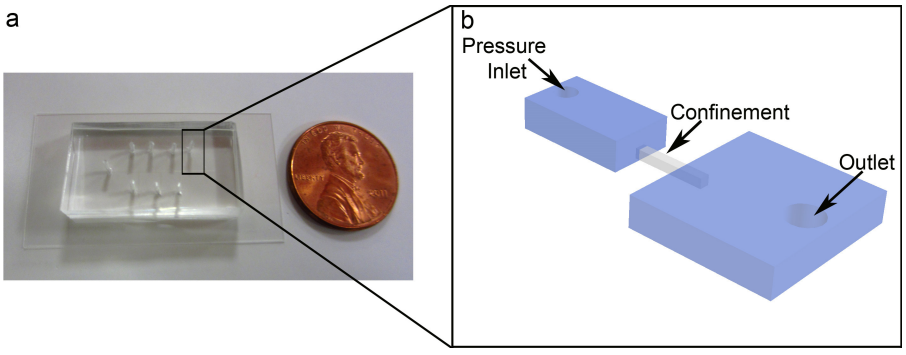

Figure 1: (a) Completed microfluidic device. (b) Schematic of a section of the device where two microchannels are connected with a sub-micron confinement. Cells are loaded at the inlet and pushed through the confinement to the outlet that is open to the atmosphere.

EXPERIMENTAL

In our system, sub-micrometer confinements and microchannels were made in PDMS using replica molding. A 3-inch master silicon wafer was patterned using electron beam and photolithography, respectively. The height of the confinement was 700 nm and the width varied from 0.75 μm to 2.0 μm. The confinement height was determined by patterning 700-nm-thick chromium metal film. The master wafer was used to make 5:1 (w/w) PDMS polymer chips. Changes in polymer base to crosslinker ratio from regular 10:1 ratio offered a firmer PDMS structure. PDMS was poured onto the wafer, heat-cured, and pealed off from the master. Chips were permanently bonded to microscope coverslips using an oxygen plasma treatment. The fabrication process is shown in Figure 2.

E. coli and *S. aureus* were cultured overnight at 37 °C in lysogeny broth (LB)-ampicillin, and in LB, respectively. Cultures were diluted 2X in LB to lower their concentration to prevent the large channels from clogging rapidly and to aid with visualization. Applied pressure was increased in 0.25 bar increments up to 5.0 bars using a Fluigent MFCS pressure-driven flow controller connected to a compressed air tank, and cell transport was monitored using brightfield and fluorescence microscopy.

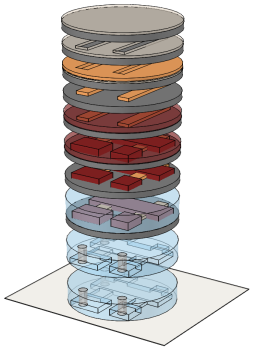

Figure 2: Fabrication schematic of a PDMS microfluidic device. 1) PMMA is spun on a Si wafer. 2) Constrictions are defined with e-beam lithography and developed. 3) Chromium metal is sputtered. 4) Metal lift-off is carried out by using acetone to remove PMMA underneath the metal. 5) Positive photoresist (AZ4620) is spun onto the wafer. 6) Microchannels are aligned and created with photolithography. 7) PDMS is molded, heat-cured, peeled off and access holes are drilled. 8) PDMS devices are bonded to glass coverslips using oxygen plasma etching.

RESULTS AND DISCUSSION

A major challenge of this project is that the minimum dimension of bacterial cells varies dramatically: 0.5-1.5 μm for *S. aureus* and 1.1-1.5 μm for *E. coli*. This necessitates numerous experiments to determine threshold values. Bacterial cultures were grown until stationary phase to maintain the cells in the same growth condition, keeping their size as uniform as possible. It is previously shown that the size of bacteria vary with respect to the growth phase bacteria are in [3]. Our preliminary results show that *S. aureus* squeezes into 700 nm tall, 1.5 μm wide constrictions starting at 0.75 bar, but requires only 0.40 bar to enter 2.0 μm wide constrictions (Figure 3). *E. coli* typically required 0.70 bar to enter 700 nm tall, 1.5 μm wide constrictions, and move through easily with 1.25 bar applied (Figure 4). Further analysis is needed to effectively remove microorganisms using sand-filtration processes.

CONCLUSION

In this study, *E. coli* and *S. aureus* were flowed through the same confinement to determine the pressure profile for each microorganism. Bacterial behavior in confinements depends on several factors: cell morphology, cell wall structure, pressure difference across the confinement, confinement material, and the size of the confinement. Our future work will include passivation of the channels to simulate soil properties and varying the Young's modulus of the confinement material.

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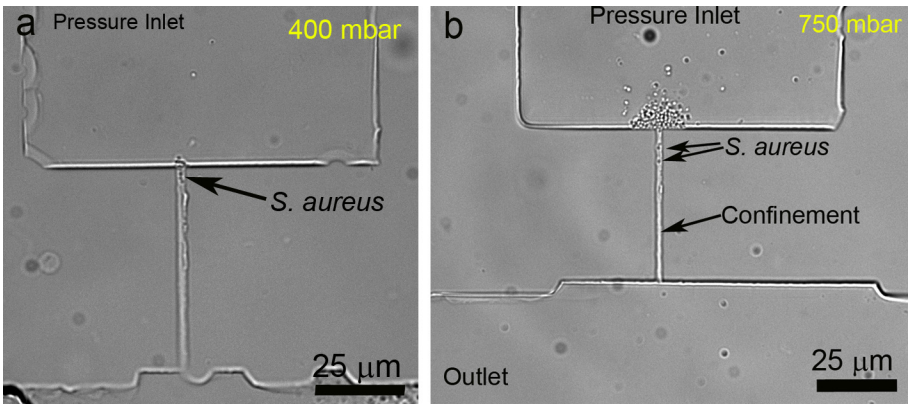

Figure 3: Brightfield micrographs of (a) S. aureus entering a 700 nm tall, 2.0 μm wide confinement using 0.40 bar, (b) S. aureus entering a 700 nm tall, 1.5 μm wide confinement with 0.75 bar applied.

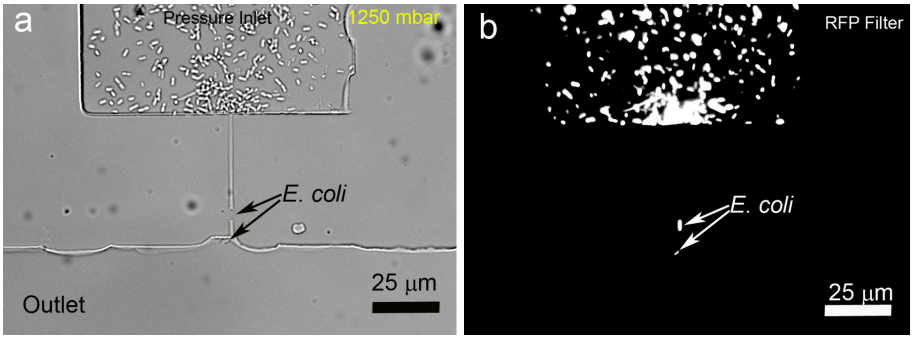

Figure 4: (a) Brightfield and (b) red fluorescent protein filtered microscope images of the same device with 700 nm tall, 1.5 μm wide confinement. E. coli was seen at the outlet region after applying 1.25 bar.

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CONTACT

* E.D. Goluch; phone: +1-617-373-3500; e.goluch@neu.edu