DEVELOPMENTS TOWARDS INTEGRATED ACOUSTIC CELL TRAPPING AND PCR

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ABSTRACT

Presented here is the first acoustic trapping of particles within a microdevice designed specifically for performing polymerase chain reaction analyses. External piezoelectric transducers were used to trap both live *Escherichia coli* cells and fluorescently labeled particles using frequencies between 6 and 11 MHz, depending of the device and desired nodal arrangement. PCR analysis of intact bacterial cells was performed using only a heat lysis step to liberate genomic DNA for amplification. As a proof of principle, PCR product was generated that is specific to the maltoporin gene in the *E. coli* genome. Given that we demonstrate trapping of cells and particles within PCR-capable acoustic resonators, and demonstrate successful single-step cell lysis and PCR, this work signifies important first steps towards an integrated microdevice for online sample enrichment followed by PCR analysis for applications such as detection of food-borne pathogens.

KEYWORDS: acoustic trapping, ultrasonic, microchip, polymerase chain reaction, infrared-mediated PCR, pathogen detection, food safety, *Escherichia coli*

INTRODUCTION

Bacterial food-borne pathogens such as *Escherichia coli* pose a significant threat to consumer health. Screening of the food supply using polymerase chain reaction (PCR) assays can help limit deadly outbreaks. However, PCR assays are time-consuming and costly, limiting their widespread implementation. Improvements in sample processing in PCR assays for food-borne pathogens is needed. This work lays the foundation for a method of on-line cell enrichment via acoustic trapping followed by rapid, infrared-mediated PCR to detect pathogens in dilute wastewater samples. Previous studies have demonstrated efficient trapping of cells in glass microdevices[2,3], single-step cell lysis and PCR[4]. To our knowledge, these two processes have never been combined in a single chamber, which would simplify the microchannel architecture significantly and facilitate automation.

THEORY

The polymerase chain reaction offers a well-known sensitivity and selectivity for target DNA sequences – traits that make it well suited to detecting a small number of bacteria in milliliters of sample[1]. Short oligonucleotides, primers, are used to target select regions of DNA sequence. A thermostable DNA-dependant DNA polymerase such as Taq polymerase extends primers bound their target, and after several heat-mediated dissociation and re-association steps (thermal cycling) up to a billion copies can be made of a single DNA strand. Multiple PCR reactions can be combined, or multiplexed, to identify a range of different pathogens.

Ultrasonic standing waves within microdevices create low pressure nodes that can be used to manipulate and trap micron-scale particles such as cells[5]. When the width of a microdevice channel or chamber matches a multiple of the half wavelength of an acoustic wave ($n*\lambda/2$) then a stable acoustic waveform is established. Such agreement between geometry and applied frequency are called resonant modes. When the acoustic energy is localized to a small area, on the order of tens of microns, particles can be aggregated and trapped while fluid flows around them in the microchannel.

EXPERIMENTAL

To demonstrate acoustic trapping of *E. coli* cells, we used a 10 MHz piezoelectric transducer coupled to a rectangular glass capillary with a vertical inner dimension of 200 μm[2]. The piezoelectric transducer was mounted to a printed circuit board, encapsulated in epoxy, and sanded to form a flat surface to make good contact with the exterior of the glass capillaries used[2]. A solution of fluorescently stained *E. coli* bacteria was flowed through the capillary device using a syringe pump. The transducer was actuated at frequencies ranging from 3 to 15 MHz, including 10.8 MHz for a triple node trapping arrangement. The region above the transducer was imaged using a compound microscope equipped with a with a fluorescent light source, FITC filter set and CMOS camera.

A second device, in effect a PCR-capable acoustic resonator, consisted of a 6 MHz piezoelectric transducer coupled to a microchip designed for rapid, infrared-mediated PCR. The PCR microchip was fabricated from borosilicate glass using conventional wet chemical etching[6], with a reaction chamber volume of 270 nL and dimensions as described in figure 1. During PCR, these devices have shown temperature ramp rates averaging −24.9 ºC/s for cooling and 17.1 ºC/s for heating[6] – values that are an order of magnitude faster than conventional thermal cyclers. The transducer was clipped to the entire bride region and glycerol was used as an ultrasound coupling medium. Solutions of fluorescent particles were introduced to one chamber of the microchip via a syringe pump. The chamber was imaged using a compound microscope equipped with similar optics to that used for the first device.

To simulate thermal cycling of trapped cells, we employed whole cell PCR (i.e. direct addition of intact *E. coli* cells to PCR reaction). Conventional PCR reaction mixtures were spiked with 250 nL of a turbid *E. coli* cell suspension to

serve as template. The cell suspension was prepared by harvesting one loop-full of cells from a confluent agar culture plate and suspending the cells into 1 mL of phosphate buffered saline (PBS). PCR primers targeting the LamB (maltoporin) gene in the *E. coli* genome were used to generate a specific 365-bp product. Heating-block thermocycling was performed in a Bio-Rad MyCycler thermal cycler. A 10-minute hold at 95 ºC prior to PCR thermal cycling was used to thermally lyse cells and activate the AmpliTaq Gold polymerase. Following the hot start/thermal lysis step, PCR was performed with 30 cycles of (10 seconds at 95 ºC, 10 seconds at 55 ºC, 10 seconds at 72 ºC) followed by a post-cycling extension step for 3 minutes at 72 ºC. Post-PCR analyses were performed via microchip gel electrophoresis using the DNA 1000 kit for the Agilent 2100 Bioanalyzer.

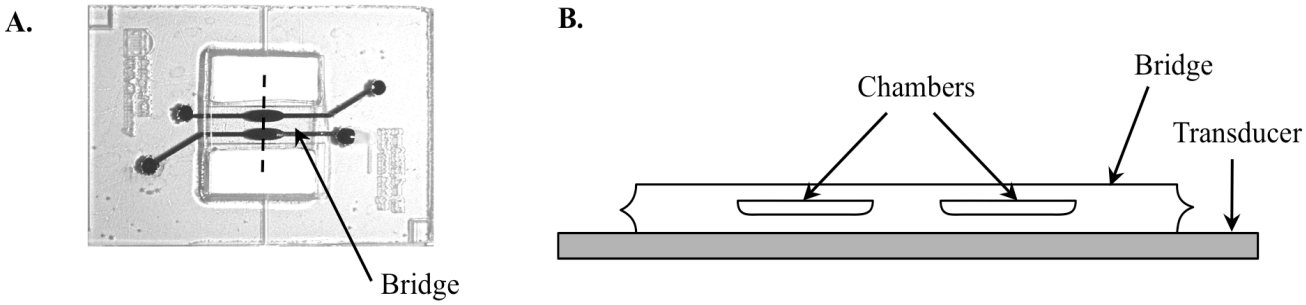

Figure 1: A. Photograph of the bridge PCR chip used in this work. Dimensions: 20 mm x 30 mm. Bridge region is 4 mm wide and chambers are 115 µm deep with ~115 µm of glass above and below. B. Cross-sectional view of the bridge region (along dotted line in panel A) that shows placement of the piezoelectric transducer.

RESULTS AND DISCUSSION

Trapping of *E. coli* was demonstrated at a variety of frequencies and flow rates in the glass capillary device. Figure 2A shows a trapped bolus of *E. coli* cells above the transducer in the glass capillary device. Cell trapping was achieved at flow rates as high as 50 µL/min. The PCR microchip was used to trap 2 µm fluorescently-tagged polystyrene beads as shown in figure 2B. Flow rates used for trapping in this PCR device were between 2 and 10 µL/min.

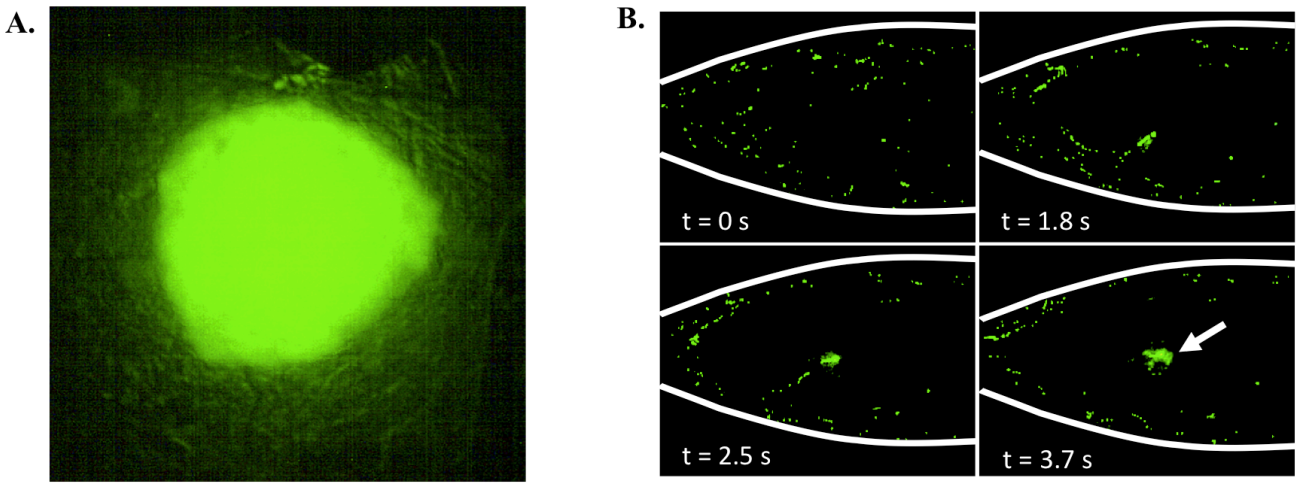

Figure 2: A. Image of trapped E. coli cells (stained with Syto 9 dye) in a capillary above a miniature transducer. Viewed through 20X objective. B. Still frames from video of focusing 2 µm fluorescently tagged beads in a bridge PCR chip. Viewed under 5X objective. White lines demark chamber walls and the white arrow in the fourth frame indicates the bolus of trapped particles.

Whole *E. coli* cells were shown to be an effective template for conventional PCR, which strongly indicates that trapped cells can be combined with PCR reagents and thermal cycled directly in the trapping/PCR chamber. Figure 3 shows overlaid traces from microchip gel electrophoresis analysis of post-PCR samples. The primary cell suspension and a 100-fold dilution both yielded the desired PCR product, thereby confirming that *E. coli* genomic DNA was present and adequately liberated from the cells by the heat lysis step.

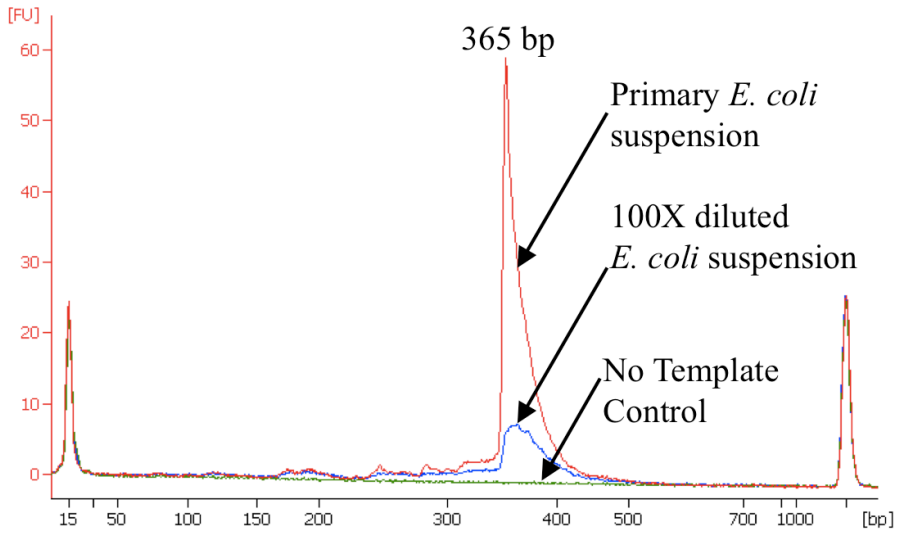

Figure 3: Electropherograms depicting successful whole-cell PCR, where intact E. coli cells were used as PCR template. The 365 bp product is generated by primers specific for the maltoporin gene in the E. coli genome.

CONCLUSIONS

Here we demonstrated successful trapping of a potential food-borne pathogen, *E. coli*, in a glass capillary under flow. A microchip designed for non-contact PCR was shown to be an acoustic resonator capable of trapping particles under flow. Although not acoustically-trapped, a whole cell PCR amplification successfully produced a PCR product that is diagnostic for *E. coli*, laying the groundwork for integration This work is a precursor to rapid and specific pathogen detection by acoustic trapping of cells for on-line PCR assays.

ACKNOWLEDGEMENTS

The authors would like to thank Lockheed-Martin for funding support and Dr. Jessica Norris of MicroLab Diagnostics for helpful conversations and technical advice.

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