Published in final edited form as:

Anal Chem. 2009 May 1; 81(9): 3523–3528. doi:10.1021/ac900060r.

Integrated Capture, Concentration, PCR, and Capillary Electrophoretic Analysis of Pathogens on a Chip

Nathaniel Beyor[†], Lina Yi^{‡,§}, Tae Seok Seo^{‡,||}, and Richard A. Mathies^{*,†,‡}

[†]UCSF/UC Berkeley Joint Graduate Group in Bioengineering, UC Berkeley

[‡]Department of Chemistry, UC Berkeley

[§]Cornell/Rockefeller/Memorial Sloan-Kettering Tri-Institutional Training Program in hemical Biology, Korea Advanced Institute of Science and Technology, Daejeon, Korea

^{||}Department of Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology, Daejeon, Korea

UCSF/UC Berkeley Joint Graduate Group in Bioengineering and Department of Chemistry, University of California, Berkeley, CA 94720, USA

Abstract

A lab-on-a-chip system for pathogen detection is presented that integrates cell preconcentration, purification, PCR, and capillary electrophoretic (CE) analysis. The microdevice is comprised of micropumps and valves, a cell capture structure, a 100 nL PCR reactor, and a 5-cm long CE column for amplicon separation. Sample volumes ranging from 10 to 100 μ L are introduced and driven through a fluidized bed of magnetically constrained immunomagnetic beads where the target cells are captured. After cell capture, beads are transferred using the on-chip pumps to the PCR reactor for DNA amplification. The resulting PCR products are electrophoretically injected onto the CE column for separation and detection of *E. coli* K12 and *E. coli* O157 targets. A detection limit of 0.2 cfu/ μ L is achieved using the *E. coli* O157 target and an input volume of 50 μ L. Finally, the sensitive detection of *E. coli* O157 in the presence of K12 at a ratio of 1:1000 illustrates the capability of our system to identify target cells in a high commensal background. This cell capture-PCR-CE microsystem is a significant advance in the development of rapid, sensitive, and specific lab-on-a-chip devices for pathogen detection.

Keywords

lab-on-a-chip; pathogen detection; cell capture; PCR; capillary electrophoresis

INTRODUCTION

Continuing progress in lab-on-a-chip development has pushed this technology into a wide array of new applications.¹ The advantages offered by these systems -speed,¹⁻³ sensitivity,⁴ automation,^{5,6} and portability⁷ - have been demonstrated repeatedly since the initial embodiment of a microfluidic chip was presented by Manz *et al.* in 1991.⁸ Born in analytical chemistry labs, initial devices focused on purely analytical techniques. Recent developments have proven the technology useful in biological diagnostics, and a research trend towards

*Corresponding author: Department of Chemistry, MS 1460, University of California, Berkeley, CA 94720. Phone: (510) 642-4192, fax: (510) 642-3599, email: E-mail: rich@zinc.cchem.berkeley.edu.

integrating sample preparation on-chip has been promising.⁹⁻¹² Pathogen detection is a particularly appropriate application for lab-on-a-chip technology because of the need for portability and a rapid response time.^{13, 14}

The threat of infectious disease is increasing dramatically as bacteria develop new drug resistances,^{15, 16} bioterrorism looms,¹⁷ and contaminated food reaches the public.^{18, 19} With these threats comes a mounting need for rapid and accurate diagnostics. Current standards for pathogen detection rely on culture plating and microscopy technologies.¹³ These methods, while accurate and sensitive, are slower and provide less information relative to current molecular diagnostic techniques such as ELISA,^{20,21} PCR,²² and microarrays.²³ Research efforts in lab-on-a-chip pathogen diagnostics have focused on incorporating these and other molecular diagnostic techniques on a microfluidic platform.

Past microfluidic systems dedicated to pathogen detection have often incorporated PCR as an amplification technique for high sensitivity analysis. Lagally *et al.* presented a portable system integrating PCR and CE capable of detecting as few as 2-3 cells.⁷ This experimental demonstration proved it capable of identifying both methicillin-sensitive and methicillin-resistant *S. aureus*, answering a critical need in pathogen detection. However, this system falls short in its ability to analyze real-world samples larger than 200 nL; thus a purification and preconcentration technique is necessary prior to the analysis. Liu *et al.* developed a microfluidic system capable of detecting *E. coli* from whole blood.¹¹ This device incorporated an immunomagnetic preconcentration and purification step which allowed 1 mL of sample to be analyzed directly on the chip. While the sensitivity was superb in respect to concentration (10^3 cfu/mL), at least 10^3 target cells were necessary for positive detection. For certain diagnostic needs, such as the detection of *E. coli* O157 where as few as 10 bacterial cells can result in infection, this detection limit is too high.²⁴ More recently, Kaigala *et al.* published a microfluidic device capable of detecting viruses directly from a clinical sample.²⁵ With a demonstrated detection limit of 1-2 viral copies, this system was used to rapidly detect the presence of BK virus from human urine samples. While the detection limit is impressive, this device is only viable for applications where the pathogen concentration is high and no PCR-inhibiting contaminants are present in the sample.

The next generation lab-on-a-chip pathogen detection system will incorporate the sensitivity of the Lagally and Kaigala systems, with the purification and preconcentration techniques of the Liu device. For highly virulent pathogens, such as *E. coli* O157, the presence of only a few cells must be detected even from a large sample volume. Furthermore, sample contaminants such as heme from blood or harmless cells must be washed away to prevent downstream PCR inhibition and microfluidic channel clogging.

The work presented here addresses the need for a highly sensitive pathogen detection microdevice with integrated sample preparation. Following on previous work optimizing cell capture structures, we use a fluidized bed of immunomagnetic beads to isolate target cells from a sample input.¹⁰ After cell capture, the bead-cell duplexes are hydrodynamically transferred to an on-chip PCR reactor for target amplification. Finally, the PCR products are injected onto a CE column and detected using laser-induced fluorescence. By integrating these three components on a single chip, we reduce the possibility of contamination during the sample analysis, increase coupling efficiency, and automate the analysis process. This structure has enabled the successful detection of pathogens at a very low level in a fully integrated microdevice.

EXPERIMENTAL SECTION

Chip Design and Fabrication

The integrated cell capture-PCR-CE microdevice shown in Figure 1 is constructed using a four layer glass-glass-PDMS-glass stack.²⁶ Each chip has two bilaterally symmetrical devices. The fluidic system incorporates three 32 nL dead volume microvalves on the fluidic side to form a microfluidic pump, a 4.1 cm long capture structure with a system of bifurcating channels, a 100 nL PCR reactor enclosed by PDMS valves, and a 5 cm (effective length) CE channel. The valving channels are enclosed using a PDMS-glass sandwich on the upper surface on the device. The cell capture, PCR, and CE channels are enclosed by thermally bonding the glass fluidic wafer to a glass resistance temperature detection (RTD) wafer, which has metal features for temperature sensing during PCR.

To form the fluidic layer, channels are etched to a depth of 38 μm on both sides of a 100-mm Borofloat wafer (500 μm thick), defining the PCR reactor and CE channels on one side and the microvalves on the other. A second wafer of the same dimensions is also etched to 38 μm for the manifold layer, which is used to enclose the PDMS valving channels. As previously described, the etching process uses an amorphous silicon hard mask with features defined through photolithography. Concentrated hydrofluoric acid (49% HF) is used to etch the glass at an average rate of 7 $\mu\text{m}/\text{min}$. This fluidic wafer is drilled with 500 μm via holes for solution transfer from one side of the wafer to the other, 1.1 mm holes for fluidic access, and 2.1 mm holes for access to RTD leads.

The RTD wafer is fabricated from a 100-mm Borofloat wafer (500 μm thick) coated on a single side with 200 $\text{\AA}$ of Ti and 2000 $\text{\AA}$ of Pt through a sputtering process^{27, 28}. Using photolithography, features are defined and etched using a 90 $^{\circ}\text{C}$ aqua regia bath. The temperature sensing element is 30 μm wide and the leads are 300 μm wide. The RTD and fluidic wafers are aligned and thermally bonded in a vacuum furnace at 670 $^{\circ}\text{C}$ for 6 hours.

Bead Preparation

Two types of superparamagnetic polystyrene bead systems were used for on-chip immunomagnetic cell capture. For *E. coli* K12 isolation, biotinylated polyclonal antibodies specific to *E. coli* (ab20640, Abcam Inc. Cambridge, MA) were conjugated to Dynabeads® M-280 Streptavidin (Invitrogen #112.05D). Based on the commercial protocol from Invitrogen, 4×10^6 beads were washed three times with 100 μL phosphate buffered saline solution (0.1 M PBS; Gibco 10010 PBS pH 7.4). Subsequently, the beads were incubated with 1.538 μg antibodies for 30 minutes in a rotator (Dyna Sample Mixer, #94701) at 20 rpm in 100 μL of PBS. Finally, beads were washed three times in PBS, once more in a 0.1% w/v solution of bovine serum albumin (BSA, 0.1M PBS) and resuspended in a volume of 20 μL PBS. For *E. coli* O157 isolation, a commercial bead system (Dynabeads anti-*E. coli* O157, Invitrogen #710.03) was used following a modified protocol. Stock solution was analyzed in a flow cytometer to determine the bead concentration to be 1.5×10^5 beads/ μL . 25.8 μL (4×10^6) beads were washed (3x) in PBS, once in a BSA solution and resuspended in 20 μL of PBS for subsequent use in on-chip cell capture experiments.

Cell Preparation

Two strains of *E. coli* were used in this experiment: *E. coli* K12 MG1655 (American Type Culture Collection, ATCC #700926) and nontoxicogenic *E. coli* O157 NCTC 12900 (ATCC 700728). Cells were grown overnight at 37 $^{\circ}\text{C}$ in a shaking incubator using a Tryptic Soy Broth medium (TSB, Hardy Diagnostics K131). *E. coli* K12 cells were transformed with a 3.9-kb pCR 2.1-TOPO vector (Invitrogen) to confer ampicillin resistance, and 1 $\mu\text{g}/\text{mL}$ of this antibiotic was added to the TSB medium for this growth. *E. coli* O157 was grown without

antibiotic resistance and frequently tested for contamination using PCR. After overnight growth, cells were washed (3x) in 2 mL PBS. Cell concentration was determined through OD₆₀₀ measurements using a calibration curve generated through serial dilution and culture plate colony growth.

PCR Reagents and Thermal Cycling

PCR amplification is performed on each *E. coli* target. Primers specific to the KI#128 island on the K12 genome (forward primer: 5' - TTC GAT TAC ACG GAG TGC TGG GAA - 3' and reverse primer: 5' - CGT TGA TTT GCC GTT CCA TGT CGT - 3'; 259 bp product) and the OI#43 island on the O157 genome (forward primer: 5' - TGG CAG GAA GAG AGT GAC AGG - 3' and reverse primer: 5' - GGC CTT ACC CGT GAA CAG TA - 3'; 191 bp product) were designed to prevent any cross-amplification between strains.²⁹ Forward primers were labeled with a 6-FAM dye on the 5-prime end (Integrated DNA Technologies). Off-chip, multiplex PCR was performed regularly to check for contamination between cell cultures. The PCR cocktail used for both on- and off-chip analysis includes 12.5 μ L Qiagen HotStarTaq Master Mix (400 μ M each dNTP, 3 mM MgCl₂, 0.1 units/ μ L Taq), 0.5 μ L forward and reverse primers (final concentration 400 nM), 0.5 μ L BSA solution (25 mg/mL in H₂O), 0.5 μ L surfactant solution (0.5% Tween 80), 0.5 μ L additional Taq polymerase (2.5 units, Qiagen HotStarTaq), and 10.5 μ L H₂O.

The thermal cycling protocol begins with a 10 minute 95 °C hot start step to activate the Taq polymerase, followed by 35 cycles of 94 °C denaturation (10 sec), 60 °C annealing (30 sec), and 72 °C extension (30 sec) steps. A 1-cm square heater (Minco) is mounted externally to facilitate thermal cycling and driven by 10 V power supply. The cycling protocol is mediated through a Labview program written in-house using a proportional, integral, differential (PID) control. Heating rates of 5 °C/sec and cooling rates of 3 °C/sec were achieved. Cooling was facilitated using blown air from the laboratory supply during the annealing step and controlled with a solenoid valve. The total reaction time is 73 minutes.

Valve Instrumentation

The valves and pumps in the cell capture-PCR-CE microdevice are operated using a Labview controlled pneumatic control system (more information can be found at <http://zinc.cchem.berkeley.edu/protocols>). The pneumatic system consists of a bank of solenoid valves, a diaphragm pump (Thomas #7011), and a PCB-based circuit that takes TTL input signals from the Labview control. Individual solenoid valves switch between a -54kPa vacuum and 19 kPa pressure to open and close their respective PDMS valves on the microdevice.

Instrumentation for CE and Detection

The glass channels are pretreated for 5 minutes with a 50% dynamic coating in methanol (The Gel Co., San Francisco, CA, DEH-100) to minimize electroosmotic flow. A 4% w/v linear polyacrylamide (LPA) separation matrix in 1x Tris Taps EDTA (TTE) buffer is used for CE separations. Sample, waste, cathode, and anode wells are filled with 1x TTE buffer for connection to external platinum electrodes. After on-chip thermal cycling, PCR products are electrophoretically injected from the sample to the waste using a 440 V/cm field, and then injected down the CE column from cathode to anode with a 270 V/cm field. During the injection, a 110 V/cm back-bias is applied from the sample and waste to the cathode. The 6-FAM dye attached to the forward primer enables detection of double stranded PCR amplicons as they migrate down the channel using laser-induced fluorescence (LIF). The Berkeley confocal rotary scanner is used to provide LIF detection.³⁰

Chip Loading and Operating Procedure

First, the LPA sieving matrix is loaded by hand from the anode up through the injection arm using a syringe. The anode, cathode, and waste wells are filled with 1x TTE buffer to prevent gel evaporation and the subsequent introduction of bubbles into the CE channels. With the micropump and the sample well valves open, acetonitrile (ACN) is drawn by vacuum from the capture well to the sample well. ACN is then pipetted into the sample well and then drawn through the PCR chamber with only the sample and waste valves open. All valves are held open to eliminate any bubbles in the system through the gas permeable PDMS membrane. Next, a 1% w/v BSA solution in PBS is drawn through the capture channels and PCR reactor in the same manner as the ACN. A 10 minute incubation allows the BSA to block nonspecific bead adhesion to the channels. 0.1 M PBS is then drawn through the channels as above to serve as a running buffer for cell capture.

Once the chip has been primed and loaded with buffer, the capture process begins by introducing 2 μL of beads (4×10^5 beads total) into the capture well. The beads are hydrodynamically driven to the capture structure using the on-chip micropump (3-step cycle, 300 ms per step) and immobilized after the first channel bifurcation using a $0.2 \times 0.4 \times 0.105''$ nickel-plated neodymium magnet (All Electronics, Van Nuys, CA, MAG-74). Allowing the beads to stack into a tight bolus after each channel bifurcation is critical to establishing equal bead distributions across all parallel capture channels. Once the two boluses are formed, the pumping is stopped and the magnet is moved to position past the second bifurcation. The process is repeated one more time to establish bead boluses of roughly equal sizes in each of the eight parallel capture channels. While the magnet is left in place over the bead boluses, the sample solution containing target cells is then added to the capture well and pumped through the capture structure using a 9-step pumping protocol that incorporates three 'flutter' steps (100 ms each).¹⁰ The flutter steps serve to pass the sample solution over the beads multiple times while maintaining a bulk solution flow in one direction. 20 μL of PBS is then rinsed through the capture channels and beads to eliminate contaminants from the system. All waste flows out to the sample well and never enters the PCR chamber, preventing any potential contamination. Finally, PBS is added to the capture well and the entire bead bed is pumped into the PCR chamber by closing the sample valve, opening the waste valve, and placing the magnet above the reactor.

To begin the PCR process, 10 μL of PCR cocktail is added to the capture well and pumped across the beads with both the waste and sample valves open, replacing the PBS as the running buffer in the system. It is critical that the pumps always run in this forward direction to prevent EDTA transfer from the injection arm to the PCR reactor; the presence of EDTA dramatically inhibits PCR. The magnet is removed, the heater is attached, and the chip is connected to the thermal cycling system. The PCR thermal cycling protocol follows as described above with all valves held closed at 28 kPa.

After the thermal cycling process is complete, the chip is transferred to the Berkeley confocal rotary scanner for CE and LIF detection of the PCR amplicons. Prior to CE, the sample valve is opened and closed 20 times. This is necessary because the thermal cycling process denatures the surface proteins on the beads, causing them to agglutinate. The valve cycling serves to hydrodynamically stir the beads and release PCR products from the agglutinated mass. With only the sample valve held open, the PCR products are injected onto the CE column and detected by LIF as described above. After each experiment, all channels are washed with piranha (4:1 H_2SO_4 : H_2O_2), water, and ACN to prevent run-to-run carryover contamination.

RESULTS AND DISCUSSION

Channel Design

Previous studies using multichannel capture structures and off-chip PCR-based quantitation have demonstrated excellent capture efficiencies.¹⁰ The challenge with the cell capture-PCR-CE microdevice was to integrate this capture structure with on-chip PCR-CE systems previously developed in our laboratory.^{2, 7, 27, 28} The basic operation of the system is: (1) capture cells on immunomagnetic beads, (2) transfer bead-cell complexes to the PCR reactor, (3) transfer PCR reagents into the reactor and perform thermal cycling, and (4) inject PCR products onto CE column for analysis. Three critical design needs were considered. First, the components must be integrated using hydrodynamic micropumps. Second, the channel design must direct all waste away from the PCR reactor to prevent contamination. Third, the fluid path must allow for beads and PCR reagents to be introduced into the reactor.

The most direct method to couple the cell capture and PCR components is to use on-chip micropumps. As shown previously, the pulsatile flow and flutter steps generated by the micropumps are critical for optimized capture efficiency.¹⁰ The micropump design requires a three-layer glass-PDMS-glass channel (shown in blue in Figure 1) to prevent beads from getting trapped in the valve seats. Four layer designs incorporating two via holes were tested but did not allow for efficient bead transfer. Initially, the system was tested using a three layer capture structure as well, since that was the device that had demonstrated optimized capture efficiency in the past. Channel deformations during the high temperature glass bonding process prevented us from using this channel design for the integrated cell capture-PCR-CE system. Therefore, the micropumps are formed using a three-layer channel structure, and then coupled to a glass-glass channel capture structure (shown in red in Figure 1) with a 500 μm diameter via hole.

In order to direct waste away from the PCR reactor, the channel design incorporates an intersection in the fluid path. During the cell capture and rinse process, the waste valve remains closed while the sample valve is left open, preventing any solution from entering the PCR reactor. Uncaptured cells, sample solution, and rinse solution are directed to the sample well by the micropumps and aspirated. PCR reagents are introduced into the reactor with the micropump after the capture process. During this process, the highly viscous sieving matrix acts as a passive valve in the injection arm, preventing any hydrodynamic flow into the CE column. As mentioned above, it is critical that the sieving matrix does not enter into the PCR reactor, as the EDTA will inhibit the polymerase. Thus, the injection arm is placed downstream of the reactor, and the pumps are only run in one direction.

E. coli K12 and O157 Detection Limit

The detection limit of the cell capture-PCR-CE system was first tested using *E. coli* K12 as a target in a sample volume of 10 μL . Here we define detection limit as the lowest input pathogen concentration sample that produces a positive signal. Immunomagnetic beads coupled to polyclonal antibodies specific to *E. coli* were used for the capture process. Primers specific to the *E. coli* K12 genome were used for PCR. Shown in Figure 2, successful detection was demonstrated for *E. coli* K12 concentrations of 1000, 100, and 10 cfu/ μL , corresponding to a total cell input of 10000, 1000, and 100, respectively. At lower concentrations no cells were detected by the system, and repeated negative controls showed no false positives. The 10 μL input volume introduces detection limitations due to pipetting loss and non-specific cell adhesion; lower detection limits were achieved using larger input volumes (see below).

During initial trials, performed without the inclusion of surfactant in the PCR reagents, we observed intermittent success. Observations of the reactor after thermal cycling showed that

the beads had aggregated during the thermal cycling process due to antibody denaturation from the high temperatures necessary for PCR. We hypothesize that bead agglutination inhibits sufficient reagent and template diffusion during the PCR process, preventing adequate amplicon production and/or release. After the addition of surfactant and a post PCR bead stirring via repeated sample valve actuation, we were able to achieve consistent results. Off-chip PCR studies verified that surfactant addition did not significantly inhibit PCR efficiency or promote cell lysis.

The limit of detection of the system was further tested using *E. coli* O157 as a target. System operation was identical to the experiments performed with the K12 target, except commercial beads for O157 capture and primers targeted against the O157 genome were used. Shown in Figure 3, the limit of detection was found to be the same for *E. coli* K12 and O157, with successful detection demonstrated at 100 and 10 cfu/ μ L corresponding to 1000 and 100 cfu input in 10 μ L.

Both experimental traces show small non-specific peaks in the 10 cfu/ μ L runs. These excess peaks were only present at low target concentrations. PCR reaction kinetics at these template concentrations increase the possibility of false amplifications, resulting in nonspecific product generation. Since the PCR reaction was run nonquantitatively for 35 cycles, these nonspecific products amplify to a detectable level. At higher template concentrations the reaction kinetics favor the amplification of the desired genetic target, resulting in a single amplicon peak.

The successful detection of *E. coli* K12 and O157 demonstrates the ability of the cell capture-PCR-CE system to identify different targets in a single-plex analysis. Using an input volume of 10 μ L, detection limits similar to previous results using off-chip quantitation have been demonstrated, and at 10 cfu/ μ L these results are comparable to previous microfluidic pathogen detection systems presented in the literature.^{7, 10, 11} Stochastic limits prevent the consistent detection of pathogens at lower target concentrations with a 10 μ L sample input volume.

Sample volume studies

The volume of sample solution was varied to study the detection sensitivity over a larger range of cell concentrations using *E. coli* O157 as a target. As shown in Figure 4, the input cell number was maintained at 10 cfu while the volume was varied from 10, 20, 50, to 100 μ L. No positive identification was achieved using a 10 μ L input volume. In this case, fixed tolerances in the volumes pipetted onto the chip can result in a loss of input cells. Furthermore, non-specific cell adhesion to the capture well is increased with this comparatively low sample volume and high cell concentration. Successful detection was shown with a 20 and 50 μ L sample volume, corresponding to 0.5 and 0.2 cfu/ μ L, respectively. At 100 μ L, the upper limit of tested input volumes, no target cells were detected. At this volume, the very low input cell concentration is not high enough to drive the cell capture interaction in the bead bed, and no detection is possible. Furthermore, the large flushing volume washes cells through the bed, similar to an excessive washing step.

This experiment demonstrates an unprecedented level of pathogen detection sensitivity on a microfluidic platform. Furthermore, it presents a technology capable of analyzing volumes previously too large for microfluidic systems. While Liu *et al.* have shown large volume analysis on a microdevice, their system required a total cell input of 10^3 cfu, two orders of magnitude higher than the results presented here.¹¹ Our cell capture-PCR-CE microsystem is capable of both high-sensitivity and large volume analysis while maintaining the speed inherent in microfluidic diagnostics.

Detection in a Cellular Background

To mimic the complex makeup of a real-world sample, an experiment was conducted to detect the presence of a low number of *E. coli* O157 cells in a high background of *E. coli* K12 cells. Immunomagnetic beads and PCR primers specific to O157 were used to isolate the target cells and amplify the DNA of interest. Off-chip PCR studies were performed to verify no cross-amplification of the primers designed for this study. In a 10 μ L input volume, 10 cfu/ μ L O157 and 10⁴ cfu/ μ L K12 were introduced into the cell capture-PCR-CE microdevice. The successful identification of O157 is shown in Figure 5, evidenced by the presence of a single 191 bp amplicon in the electrophoretic separation.

Identifying pathogens in a complex sample requires a purification and concentration step to remove target cells and contaminants from the input volume. This background experiment, where 10 cfu/ μ L *E. coli* O157 cells are detected in a K12 background 1000-fold more concentrated, illustrates both the sensitivity and specificity of the cell capture-PCR-CE microdevice.

CONCLUSIONS

Our cell capture-PCR-CE microdevice demonstrates a significant advancement in the field of microfluidic systems for pathogen detection. While maintaining the advantages of analysis speed and compact size, the detection sensitivity and large analysis volume shown here are previously unseen in microfluidic systems. By directly coupling the capture structure with the PCR-CE components, the possibility of contamination is minimized. Furthermore, the detection limit of 0.2 cfu/ μ L is an order of magnitude lower than other systems developed for pathogen detection^{7, 11} and was achieved while analyzing a volume that is roughly 250 times greater than devices developed by our laboratory in the past.^{2, 7, 27} Finally, the detection of *E. coli* O157 shows that this system is capable of identifying a dangerous pathogen with contemporary significance at clinically relevant sensitivities.

In the future, this system could be adapted for portable and multiplex analysis. Because the likelihood of detecting pathogens from a real-world sample is small, each PCR reaction can be multiplexed with the expectation that only one or a few pathogen targets will be present in any given sample. Nevertheless multiple separate PCR reactors are necessary, each containing a small set of primers specific to different pathogens. The best sensitivities will likely result from a single flow through capture structure with a series of different bead beds, each of which contains immunomagnetic beads that bind a chosen subset of the bacterial targets. Each capture structure is coupled to a unique downstream PCR-CE analysis system and the bead bed is distributed to its corresponding reactors through the microfluidic pump and valve networks demonstrated here. The development of rapid, sensitive, and specific pathogen detection techniques capable of analyzing multiple targets from a single sample is critical for the protection of the public at large from bioterrorism, the food supply from bacterial contaminants, and patients from drug resistant superbugs.³¹⁻³⁵

ACKNOWLEDGEMENTS

We appreciate the assistance of Robert Blazej, Teris Liu, and Nick Toriello in completing this work. All microfabrication was performed at the UC Berkeley Microfabrication Laboratory. Funding for this project was provided by the NIH (#U01AI056472).

REFERENCES

- (1). Lagally ET, Mathies RA. J. Phys. D: Appl. Phys 2004;37:245–261.
- (2). Liu P, Seo TS, Beyor N, Shin KJ, Scherer JR, Mathies RA. Anal. Chem 2007;79:1881–1889. [PubMed: 17269794]

- (3). Seiler K, Harrison DJ, Manz A. *Anal. Chem* 1993;65:1481–1488.
- (4). Ocvirk, G.; Tang, T.; Harrison, DJ. *Analyst*. Vol. 123. Cambridge, U. K.: 1998. p. 1429–1434.
- (5). Gomez-Sjoberg R, Leyrat AA, Pirone DM, Chen CS, Quake SR. *Anal. Chem* 2007;79:8557–8563. [PubMed: 17953452]
- (6). He Y, Zhang YHH, Yeung ES. *J. Chromatogr., A* 2001;924:271–284. [PubMed: 11521874]
- (7). Lagally ET, Scherer JR, Blazej RG, Toriello NM, Diep BA, Ramchandani M, Sensabaugh GF, Riley LW, Mathies RA. *Anal. Chem* 2004;76:3162–3170. [PubMed: 15167797]
- (8). Manz A, Harrison DJ, Verpoorte EMJ, Fettingner JC, Ludi H, Widmer HM. *Chimia* 1991;45:103–105.
- (9). Breadmore MC, Wolfe KA, Arcibal IG, Leung WK, Dickson D, Giordano BC, Power ME, Ferrance JP, Feldman SH, Norris PM, Landers JP. *Anal. Chem* 2003;75:1880–1886. [PubMed: 12713046]
- (10). Beyor N, Seo TS, Liu P, Mathies RA. *Biomed. Microdevices* 2008;10:909–917. [PubMed: 18677651]
- (11). Liu RH, Yang JN, Lenigk R, Bonanno J, Grodzinski P. *Anal. Chem* 2004;76:1824–1831. [PubMed: 15053639]
- (12). Tian H, Hühnera AFR, Landers JP. *Anal. Biochem* 2000;283:175–191. [PubMed: 10906238]
- (13). Forbes, BA.; Sahm, DF.; Weissfeld, AS. *Bailey and Scott's Diagnostic Microbiology*. Vol. 11th ed.. Mosby; St. Louis: 2002.
- (14). Koh CG, Tan W, Zhao MQ, Ricco AJ, Fan ZH. *Anal. Chem* 2003;75:4591–4598. [PubMed: 14632069]
- (15). abcNEWS. 2005. 'Superbug' MRSA Worries Doctors, Athletes.
- (16). Brower, J.; Chalk, P. *The Global Threat of New and Reemerging Infectious Diseases: Reconciling U.S. National Security and Public Health Policy*. Rand; Santa Monica, CA: 2003.
- (17). Grow RW, Robinson L. *Biosecurity and Bioterrorism* 2003;1:215–220. [PubMed: 15040199]
- (18). Saulo AA. *Food and Safety Technology* 2006;19
- (19). Russell, S. *San Francisco Chronicle*. San Francisco: 2006. Manure tests positive for E. coli strain that killed 3 people.
- (20). Lazcka O, Del Campo FJ, Muñoz FX. *Biosens. Bioelectron* 2007;22:1205–1217. [PubMed: 16934970]
- (21). Crowther, JR. *ELISA Theory and Practice (Methods in Molecular Biology)*. Vol. 1st ed.. Humana; Totowa, NJ: 1995.
- (22). Mothershed EA, Whitney AM. *Clin. Chim. Acta* 2006;363:206–220. [PubMed: 16139259]
- (23). Call DR. *Crit. Rev. Microbiol* 2005;31:91–99. [PubMed: 15988839]
- (24). *Bad Bug Book*. U.S. Food And Drug Administration; 2001.
- (25). Kaigala GV, Huskins RJ, Preiksaitis J, Pang XL, Pilarski LM, Backhouse CJ. *Electrophoresis* 2006;27:3752–3763.
- (26). Toriello NM, Liu CN, Blazej RG, Thaitrong N, Mathies RA. *Anal. Chem* 2007;79:8549–8556. [PubMed: 17929900]
- (27). Liu CN, Toriello NM, Mathies RA. *Anal. Chem* 2006;78:5474–5479. [PubMed: 16878885]
- (28). Toriello NM, Liu CN, Mathies RA. *Anal. Chem* 2006;78:7997–8003. [PubMed: 17134132]
- (29). Perna NT, Plunkett G, Burland V, Mau B, Glasner JD, Rose DJ, Mayhew GF, Evans PS, Gregor J, Kirkpatrick HA, Pósfai G, Hackett J, Klink S, Boutin A, Shao Y, Miller L, Grotbeck EJ, Davis NW, Lim A, Dimalanta ET, Potamouisis KD, Apodaca J, Anantharaman TS, Lin J, Yen G, Schwartz DC, Welch RA, Blattner FR. *Nature* 2001;409:529–533. [PubMed: 11206551]
- (30). Shi Y, Simpson PC, Scherer JR, Wexler D, Skibola C, Smith MT, Mathies RA. *Anal. Chem* 1999;71:5354–5361. [PubMed: 10596215]
- (31). Shah NS, Wright A, Bai GH, Barrera L, Boulahbal F, Martin-Casabona N, Drobniewski F, Gilpin C, Havelkova M, Lepe R, Lumb R, Metchock B, Portaels F, Rodrigues MF, Rusch-Gerdes S, Van Deun A, Vincent V, Laserson K, Wells C, Cegielski JP. *Emerging Infectious Diseases* 2007;13:380–387. [PubMed: 17552090]
- (32). Gerner-Smidt P, Whichard JM. *Foodborne Pathogens and Disease* 2007;4:391–394. [PubMed: 18041948]

- (33). Edwards KA, Clancy HA, Baeumner AJ. *Anal. Bioanal. Chem* 2006;384:73–84. [PubMed: 16283259]
- (34). Elston DM. *J. American Acad. of Dermatology* 2007;56:1–16.
- (35). Shi Y. *Electrophoresis* 2006;27:3703–3711. [PubMed: 16960837]

Figure 1.

Cell capture-PCR-CE microdevice that integrates a capture structure with PCR and capillary electrophoresis. Immunomagnetic beads are immobilized by an external magnet in the capture channels as sample solution is driven through the bead bed. After capture and washing, the bead-cell duplexes are pumped into the 100 nL PCR reactor, where an external heater drives the reaction. Finally, the PCR amplicons are injected onto the CE column, separated, and detected near the anode using LIF. Channels in blue are enclosed using a PDMS-glass sandwich. Channels in red are enclosed by thermally bonding the etched glass wafer to the RTD wafer. Green features delineate the Ti/Pt resistance temperature detector features.

Figure 2.

Detection limit of the cell capture-PCR-CE microdevice using *E. coli* K12 as a target. Immunomagnetic beads with polyclonal antibodies specific to *E. coli* were used to capture the cells from the sample, and primers designed against island KI#128 on the *E. coli* K12 genome were used in the PCR. The presence of the 259 bp amplicon demonstrates a detection sensitivity of 10 cfu/μL or 100 cfu in the 10 μL input sample.

Figure 3.

Detection limit of the cell capture-PCR-CE microdevice using *E. coli* O157 as a target. Commercial immunomagnetic beads specific to *E. coli* O157 (Invitrogen) were used to capture the cells from the sample, and primers designed against island OI#43 in the *E. coli* O157 genome were used in the PCR. The presence of the 191 bp amplicon demonstrates a detection sensitivity of 10 cfu/μL or 100 cfu in the 10 μL input sample.

Figure 4. Dilution study using the cell capture-PCR-CE microdevice. Using a fixed number of input *E. coli* O157 cells (10 cfu), the cell capture-PCR-CE system was tested with input sample volumes of 10, 20, 50 and 100 µL. Positive detection is demonstrated in the 20 µL and 50 µL traces, corresponding to a lower detection limit of 0.2 cfu/µL. No product peak is present in the most dilute 100 µL trace.

Figure 5. Detection of *E. coli* O157 in a background of *E. coli* K12. The ratio of O157 to K12 used in this test is 1:1000. The 10 μ L sample input contains 10 cfu/ μ L O157 and 10^4 cfu/ μ L K12.